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THE PEPTIZATION OF WHEAT FLOUR PROTEINS BY INORGANIC SALT SOLUTIONS[1]

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Introduction

Hoffman and Gortner (1927) have recently reported experiments dealing with the properties of certain protein fractions isolated from wheat flour. It was noted that the yields of the albumin and globulin fractions were not identical when a 5 per cent K_2SO_4 solution was used to replace the classical 10 per cent NaCl solution. This observation raised the question as to the nature of the process involved in the extraction of the "salt-soluble" proteins and as to the extent that such "solubility" would be affected by various anions or cations.

The physiological and biochemical committees on protein nomenclature (1908) define globulins as "simple proteins insoluble in pure water but soluble in neutral solutions of salts of strong bases with strong acids. (The precipitation limits with ammonium sulfates should not be made a basis for distinguishing the albumins from the globulins)." It will be noted that this definition is indefinite, in that no statement is made as to the concentration of the salt solution. This has been remedied in most textbooks where the definition is usually worded, "Simple proteins, heat-coagulable, insoluble in water but soluble in dilute solutions of salts of strong bases with strong acids." Even here there may well be a difference of opinion as to the concentration of a "dilute" solution, and one would gather from the definition that all salts of strong acids with strong bases would be equally suitable for extracting globulins. Apparently no one has raised the questions of what salts and what concentrations, nor are the necessary experimental data available to enable one to decide these questions.

[1] Published with the approval of the Director as Paper No. 755, Journal Series, Minnesota Agricultural Experiment Station.

In addition to these considerations the modern viewpoint of colloid chemistry has altered our views in regard to "solubility" and differentiates sharply between true solutions and peptization. Undoubtedly most of the so-called protein "solutions" are in reality colloidal sols, and accordingly their formation can be most easily interpreted if we bear in mind the phenomena that are characteristic of peptization.

With the above in mind, a series of experiments was undertaken in order to ascertain what relationships existed between the nature of the salt, the concentration of the salt solution, and the proportion of protein that could be extracted from a series of wheat flours. The results were so unexpected that it is deemed worth while to report them in detail.

Experimental

The Material.—Twelve samples of wheat flour, differing widely in origin and in physical and chemical properties, were selected from the lot used in experiments by Grewe and Bailey (1927a, 1927b). As the physical and chemical characteristics of these flours have already been reported in detail, they will not be repeated here. The reader is referred to the papers by Grewe and Bailey for all data except the percentage of crude protein in the flours and the loaf volumes. These data, taken from the above papers, are shown in Table I.

TABLE I

PROTEIN CONTENT (DRY BASIS) AND LOAF VOLUME OF THE FLOURS USED*

Lab. No.	Crude protein	Loaf volume
	%	cc.
1	9.73	1810
2	13.99	1990
3	13.07	2180
4	12.53	2130
5	14.29	2170
6	12.34	2140
7	10.61	1990
8	10.63	1970
13	10.09	2020
14	12.76	2150
15	12.49	2200
17	13.64	1940

*Data of Grewe and Bailey (1927).

The salts used were all of "analytical" purity and were purchased in original containers. The majority of the salts were used in four concentrations. Three of these concentrations—0.5 N, 1.0 N, and 2.0 N—were selected to represent more or less similar ionic concentrations of the various salts. It is obvious that a 5% solution of NaCl and a 5% solution of KCl do not represent equivalent ionic strengths, and there is no justification other than tradition for the comparative use

of such concentrations. On the other hand, we have employed certain of the salts in 5% or 10% concentrations so we may have data that are comparable with other data already in the literature.

The Method.—Six grams of wheat flour was suspended in 50 cc. of a salt solution of known concentration and the suspension shaken for 30 minutes in a mechanical shaker. The flour residue was then tightly packed in the bottom of the tube by whirling in an electric centrifuge, centrifuging being continued until the supernatant liquid was clear. The supernatant liquid was then carefully decanted into a Kjeldahl flask and a second extraction of the flour residue was carried out by adding a fresh 50-cc. portion of the salt solution, shaking for 30 minutes, centrifuging, and decanting into the same Kjeldahl flask. A third extraction was similarly made. The protein, dissolved by the three successive salt extractions, was then determined on the combined extracts by the usual Kjeldahl-Gunning procedure. Nitrogen and moisture were determined on the flour samples and the salt extraction data calculated on the moisture-free basis as per cent of total protein dissolved (i. e., peptized) by the salt solution. Duplicate extractions were made in all instances, and if the usual limit of error for a Kjeldahl determination was exceeded in the percentage of nitrogen extracted, the extractions were repeated on additional samples until satisfactory analytical agreement was obtained.

Experimental Data.—The experimental data, expressed in percentage of total protein extracted from wheat flours by the different salts in the various concentrations are shown in Tables II to V. Table VI shows the average percentage of protein extracted from the 12 wheat flours by the salts in their various concentrations. These data are reproduced graphically in Figure 1. Tables VII to X show the percentage of non-gluten protein extracted by the different salts in their various concentrations. Tables VII to X are based on the assumptions that the gliadin and glutenin are chemical entities, that together they constitute the gluten proteins, and that the values for gliadin and glutenin reported by Grewe and Bailey (1927) represent the true content of gliadin and glutenin in the flours examined. Accordingly the portion of the protein that is neither gliadin nor glutenin represents the non-gluten protein.

Discussion

It is obvious from an inspection of the preceding tables that there is a great variability in the amount of protein that can be extracted from a given wheat flour by various salt solutions of equivalent ionic

concentration. The series of KF, KCl, KBr, KI, is perhaps the most striking example. In this series the 1.0 N concentrations extracted average amounts of protein, ranging from 13.07 to 63.89, or percentages of the non-gluten protein ranging from 69.2 to 340.2.

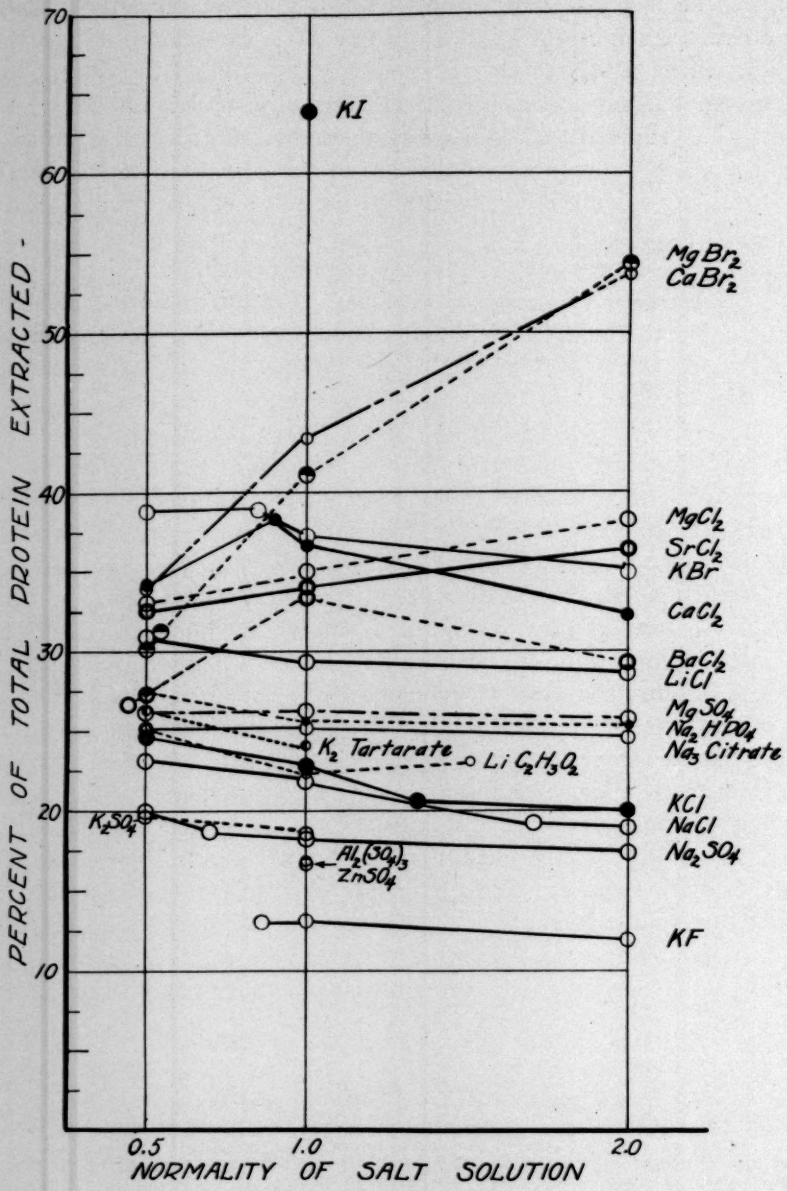

Fig. 1. Average Percentage of Protein Extracted from 12 Wheat Flours by Various Concentrations of Salt Solutions

Equally striking is the variability of the individual flours toward a single salt solution. Thus, with 1.0 N KF the range of non-gluten protein extracted from the various flours varies from 51% to 95%, with KCl from 84% to 160%, with KBr from 150% to 256%, and with KI from 266% to 391%. There is approximately 100% difference in the extreme ranges within the various flours for any given salt solution.

Certain of these data have already been discussed at length in regard to the colloidal problems which are involved (Gortner, Hoffman, and Sinclair, 1928, 1928a). In these papers the following conclusions[2] were drawn:

1. There is a pronounced lyotropic or Hofmeister series of anions arranging themmselves in the order of increasing peptizing effect of $F < SO_4 < Cl <$ tartarate $< Br < I$.

2. There is a less pronounced, but still distinct, lyotropic series of cations, in order of increasing peptization of $Na < K < Li < Ba < Sr < Mg < Ca$.

3. Hydrogen-ion concentration differences do not account for these lyotropic series. The lyotropic effects are due to the properties of the anion and cation of the salt and are observable and measurable even at constant H-ion concentration.

4. The alkali halides all cause decreasing peptization with increased salt concentration.

5. The halides of the alkaline earths, as a rule, cause increased peptization with increasing salt concentration. This is particularly noticeable for $MgCl_2$, $MgBr_2$, $SrCl_2$, and $CaBr_2$ solutions.

6. Our data show that protein "solubility" in neutral salt solutions is, in reality, protein peptization, and as such it is governed as to rate and extent by the nature of the particular anions or cations present in the salt solution.

7. Globulins are defined as "proteins soluble in dilute solutions of salts of strong acids with strong bases," hence we wish to raise the questions, *what salts, what dilutions,* and lastly, *what is a globulin?*

8. Our data show that N/1 solutions of KF extract an average of 13%, KCl 23%, KBr 37%, and KI 64% of protein from wheat flours. Which salt extracts the globulins? We do not believe that any salt used, in any one of the concentrations, extracts a chemical entity which should be designated by the term "globulin."

[2] It would be a needless duplication to reproduce here the argument from which these conclusions were drawn. The reader is referred to the cited papers for the development of the argument.

9. "Peptization" by neutral salt solutions is not "hydrolysis," for there is no increase in free amino or carboxyl groups.

10. It is pointed out that the colloid chemical viewpoint still affords the most satisfactory explanation for many of the properties of protein systems.

In addition to these conclusions there are certain observations which should interest primarily the cereal chemist. If Conclusion 8 is valid, then the question arises as to what value the cereal chemist can place upon the "salt-soluble protein" fraction. The cereal chemist rather generally uses 5% K_2SO_4 solutions to extract this fraction. An inspection of Table VII shows that this concentration of K_2SO_4 with the various flours examined gives a range of from 70% to 123% of the non-gluten protein, that is, in Flour No. 15 only 70% of the "albumins and globulins" was dissolved by the potassium sulfate solution, whereas in Flour No. 1 an appreciable amount of the gluten proteins was peptized. Somewhat similar effects are shown by 10% NaCl, the extreme ranges being from 78% to 138% of the non-gluten proteins.

The *average* values for 10% NaCl, 10% KCl, 5% Na_2SO_4, and 5% K_2SO_4; for 0.5 N and 1.0 N Na_2SO_4, K_2SO_4; and for 2.0 N NaCl, KCl, K_2SO_4, and Na_2SO_4 approximate the true values for the non-gluten proteins. However, the individual variability of the flours makes it evident that *the nitrogen extracted by a salt solution from any particular flour does not necessarily indicate the proportion of non-gluten proteins present in that flour.*[3]

If the foregoing statement be true, then it must be evident from the data presented that *the gluten proteins present in wheat flour show a pronounced variability in the ease with which they are peptized by salt solutions.* The gluten proteins of flour No. 1 show, with but few exceptions, the greatest ease of peptization, whereas the gluten proteins of flour No. 15 show, in general, the least peptization, altho there are a few striking exceptions. These exceptions are sufficiently numerous to indicate that there is an inherent difference between the behavior of the proteins of any particular sample of flour and of the proteins of another sample and that these differences are sufficiently pronounced to be easily recognizable in the effect of different salt solutions on ease of peptization. It is doubtful if these differences are attributable to constitutional differences in the proteins. In all probability they are the result of differences in the colloidal properties, such as have been demonstrated to occur in different flours. Gortner and Doherty (1918), Sharp and Gortner (1922), and Gortner and Sharp (1923) have shown

[3] Assuming that gliadin and glutenin can be determined with a fair degree of accuracy and that they constitute the gluten proteins.

that the gluten proteins of certain flours are more readily peptized by acids and bases than those of other flours. Accordingly it appears logical that similar differences in colloidal behavior should accompany treatment with neutral salt solutions. Altho we have demonstrated that such differences do occur, we are as yet unable to offer any explanation as to the reasons underlying their occurrence. As suggested by Gortner and Doherty (1918), these differences probably have their origin in the environmental factors under which the wheat was grown, and to this suggestion we wish to add that in all probability the maturity of the wheat at the time of harvest and the environmental conditions surrounding the wheat and grain subsequent to harvest may well play a rôle.

Summary

Twelve wheat flours, differing widely in type and geographical origin, have been extracted with a series of inorganic salt solutions of varying concentrations in order to study the variability in degree of peptization which exists (a) between the various salt solutions and the proteins of any one flour, and (b) between any particular salt solution and the proteins of a series of flours.

The following conclusions are drawn:

1. There is great variability in the amount of protein that can be extracted from a given wheat flour by various salt solutions of equivalent ionic concentration.

2. There is an equally striking variability of the proteins of individual flours toward a single salt solution. The amount of protein peptized by a single salt solution will vary 100 per cent in the extreme ranges for the various flours studied.

3. These differences are not dependent upon the H-ion concentration but are determined by (1) the ease of peptization of the proteins in a particular flour and (2) the specific properties of the particular anions and cations present in the salt solution used.

4. The salt-soluble protein fraction does not represent a mixture of albumin and globulin, nor does it represent the non-gluten proteins. Some salts extract only a part of the non-gluten proteins, whereas others extract very appreciable amounts of the gluten proteins. Thus, 1.0 N KF extracts an average of 69% of the non-gluten proteins, whereas an equivalent concentration of KI extracts 340%.

5. The peptization of the wheat flour proteins by inorganic salt solutions reveals the same sort of differences as does peptization with acid or alkaline solutions. It is believed that these differences are associated with the colloidal properties of the wheat flour proteins which

in turn are dependent upon heritable differences of the wheat varieties and upon environmental conditions under which the wheat was grown or that are involved in the subsequent harvesting and storage of the grain.

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TABLE II

PERCENTAGE OF TOTAL PROTEIN EXTRACTED FROM WHEAT FLOURS BY CERTAIN 10% OR 5% SALT SOLUTIONS

Salt	Concentration of solution %	Normality of solution	Flour No. 1	2	3	4	5	6	7	8	13	14	15	17	Range of flours	Average of flours
Li Acetate	10	1.515	33.4	19.4	19.8	17.8	19.3	23.9	24.3	27.9	28.8	22.8	20.0	21.3	17.8–33.4	23.23
LiCl	10	2.358	39.1	26.6	22.9	23.5	26.5	27.0	31.2	31.9	36.0	27.6	24.5	29.3	22.9–39.1	28.84
NaCl	10	1.710	26.3	17.1	16.0	16.4	16.0	18.6	18.7	21.0	23.2	18.9	16.7	21.5	16.0–26.3	19.20
Na_2SO_4	5	0.702	26.0	17.0	16.5	15.0	16.1	18.0	18.4	18.1	25.6	18.9	15.6	19.8	15.0–26.0	18.75
Na_3 Citrate	5	0.581	43.0	20.7	19.2	16.5	20.8	22.3	27.1	30.0	34.2	23.1	23.6	21.7	16.5–43.0	25.18
Na_2HPO_4	5	0.703*	35.0	19.9	17.5	15.7	18.6	16.7	25.9	30.5	33.8	22.8	24.7	23.1	15.7–35.0	23.68
KF	5	0.860	18.7	11.3	11.5	10.2	11.3	12.0	13.2	15.0	16.1	12.8	11.0	13.9	10.2–18.7	13.08
KCl	10	1.341	27.4	18.7	17.1	16.7	18.5	20.0	20.8	22.7	26.0	20.1	17.0	21.8	16.7–27.4	20.56
KBr	10	0.840	52.1	41.8	36.8	30.7	37.0	38.3	36.8	39.2	45.0	34.3	32.3	40.2	31.6–52.1	38.80
K_2SO_4	5	0.574	23.5	16.5	15.7	15.7	15.9	17.3	19.0	19.8	21.9	18.0	14.9	19.2	15.7–23.5	18.12
K_2 Tartrate	5	0.442	33.3	21.7	20.4	18.4	21.8	21.9	24.7	26.5	29.0	24.1	21.0	23.7	18.4–33.3	23.87
K_2CrO_4	5	0.515	41.0	26.7	23.1	16.0	25.8	22.1	27.3	30.6	39.0	28.3	25.1	29.2	16.0–41.0	27.85
$MgCl_2$	5	1.049	45.3	31.1	35.1	28.9	29.7	35.3	33.6	36.2	39.4	37.6	30.7	34.3	28.9–45.3	34.77
$MgBr_2$	5	0.543	39.0	29.2	28.5	25.2	26.8	28.4	33.4	34.8	38.6	30.5	28.2	31.9	25.2–39.0	31.21
$MgSO_4$	5	0.831	37.1	21.8	21.7	19.0	20.9	23.5	26.3	28.5	31.6	24.1	23.2	24.1	19.0–37.1	25.15
$CaCl_2$	5	0.901	52.5	37.5	35.3	31.4	35.3	35.9	37.6	41.6	42.7	37.2	33.4	39.2	31.4–52.5	38.30
$CaBr_2$	5	0.500	40.5	30.7	28.7	29.8	30.9	32.9	36.7	36.3	40.2	34.2	31.7	33.4	28.7–40.5	33.97
$SrCl_2$	5	0.631	41.4	30.5	29.1	26.9	28.6	29.6	31.4	31.3	37.0	30.3	27.5	32.0	26.9–41.4	31.30
$BaCl_2$	5	0.480	32.9	25.5	24.5	23.0	25.1	25.0	27.4	28.7	30.6	26.6	24.6	25.8	23.0–32.9	26.64
Average of salts	..	.....	36.18	24.40	23.19	20.89	23.42	24.67	27.04	29.00	32.07	25.90	23.46	26.60		

*Calculated as *di valent*.

TABLE III

PERCENTAGE OF TOTAL PROTEIN EXTRACTED FROM WHEAT FLOURS BY 0.5 NORMAL SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
Li Acetate	36.0	22.7	20.5	18.6	22.2	24.2	26.3	29.1	32.0	24.3	21.9	22.3	18.6–36.0	25.01
LiCl	38.5	30.4	27.1	24.7	26.7	28.0	30.4	32.5	37.1	28.8	27.5	29.3	24.7–38.5	30.08
NaCl	29.4	22.4	20.4	19.2	20.2	22.7	22.9	26.1	27.9	21.9	20.3	24.5	19.2–29.4	23.16
Na_2SO_4	27.5	18.2	17.7	15.6	17.3	19.1	20.9	21.5	24.5	20.2	16.4	21.1	15.6–27.5	20.00
$Na_3Citrate$	41.1	21.7	18.4	16.8	20.2	23.1	28.8	30.9	28.8	24.1	24.9	22.1	16.8–41.1	25.07
Na_2HPO_4*	36.7	21.8	18.6	18.5	21.3	24.2	33.9	35.5	38.8	27.9	27.5	24.6	18.5–36.7	27.44
KCl	31.2	24.0	21.9	20.4	22.3	23.3	24.5	26.3	29.8	23.9	21.0	26.8	20.4–31.2	24.62
KBr	50.7	41.8	37.8	32.3	37.7	37.5	37.3	39.9	42.1	34.4	32.9	40.9	31.7–50.7	38.77
K_2SO_4	26.6	17.9	18.2	16.3	17.3	18.9	19.0	21.5	23.3	19.9	16.3	21.0	16.3–26.6	19.68
K_2 Tartrate	36.0	22.9	24.3	19.1	23.4	26.2	29.1	30.2	30.2	24.8	25.9	24.1	19.1–36.0	26.35
K_2CrO_4	41.9	27.0	21.0	17.0	22.7	23.5	27.4	30.4	35.7	25.6	23.1	27.7	12.7–41.9	26.92
$MgCl_2$	43.3	31.7	31.6	27.5	30.1	31.0	33.6	35.3	37.8	31.4	29.4	33.4	29.4–43.3	33.01
$MgBr_2$	37.8	28.1	28.9	26.0	27.9	29.5	33.9	34.4	37.7	28.0	29.3	20.5	20.5–38.2	30.17
$MgSO_4$	36.7	23.4	24.8	20.3	23.4	25.0	27.8	27.9	33.0	25.0	23.4	23.5	20.3–36.7	26.18
$CaCl_2$	45.8	34.9	32.0	28.4	31.1	32.1	34.0	35.0	39.3	32.5	30.1	34.5	28.4–45.8	34.14
$CaBr_2$	40.5	30.7	28.7	29.8	30.9	32.9	36.7	36.3	40.2	34.2	31.7	33.4	28.7–40.5	33.90
$SrCl_2$	45.7	32.2	31.1	27.3	30.7	30.9	31.5	34.0	37.8	29.4	27.9	32.6	27.3–45.7	32.59
$BaCl_2$	36.4	24.6	25.0	21.8	24.0	25.2	28.0	28.7	33.1	28.1	24.7	27.9	21.8–36.4	27.29
Average of salts	37.88	26.47	24.89	22.20	24.97	26.52	29.22	30.86	33.84	26.91	25.21	27.23		

*Considered as *di valent*.

TABLE IV

PERCENTAGE OF TOTAL PROTEIN EXTRACTED FROM WHEAT FLOURS BY 1.0 NORMAL SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
Li Acetate	29.4	19.3	19.0	16.9	19.4	21.4	23.7	26.0	29.1	21.6	20.2	21.9	16.9–29.4	22.32
LiCl	39.3	26.8	27.5	23.9	27.0	29.1	29.9	30.3	34.0	28.1	26.3	29.7	23.9–39.3	29.32
NaCl	25.4	21.2	20.2	19.2	18.3	21.2	22.2	23.7	26.8	21.6	17.9	22.5	17.9–25.4	21.68
Na_2SO_4	25.7	16.5	16.0	14.8	15.6	17.2	18.1	18.9	22.9	18.7	14.8	18.8	14.8–25.7	18.20
Na_3Citrate	42.5	lost	17.3	15.6	18.2	20.7	29.5	31.2	34.1	22.7	23.8	20.8	15.6–42.5	25.13
Na_2HPO_4	41.5	22.1	19.4	16.6	19.4	21.6	29.9	31.4	36.5	23.3	24.0	21.8	16.6–37.5	25.62
KF	18.0	11.9	11.2	10.5	11.4	12.6	12.4	14.3	15.5	13.0	10.8	15.2	10.5–18.0	13.07
KCl	30.4	21.7	20.7	18.7	20.9	22.0	22.3	25.0	27.7	22.7	17.9	23.3	18.7–30.4	22.77
KBr	48.6	39.4	35.7	30.1	36.3	35.5	36.3	38.3	41.2	35.0	31.8	38.4	30.1–48.6	37.22
KI	73.9	69.5	60.5	55.4	64.2	66.1	61.6	63.1	68.0	63.6	56.5	64.3	55.4–73.9	63.89
K_2SO_4	26.2	16.4	16.1	14.8	15.8	17.2	18.9	20.2	23.3	19.9	14.8	19.5	14.8–26.2	18.59
K_2 Tartrate	34.6	21.7	21.7	18.4	20.2	22.3	28.7	28.1	25.0	24.1	23.6	21.1	18.4–34.6	24.12
$MgCl_2$	47.0	34.3	33.1	29.1	31.4	33.6	34.8	36.5	40.2	33.0	30.9	36.0	29.1–47.0	34.99
$MgBr_2$	46.5	40.9	38.4	32.9	38.9	38.2	42.7	42.5	46.4	37.5	37.2	39.2	32.9–46.5	40.11
$MgSO_4$	41.6	26.6	22.0	19.6	21.3	23.9	27.3	30.2	31.5	23.8	24.4	24.0	19.6–41.6	26.35
$CaCl_2$	47.2	36.7	34.1	30.9	35.5	33.0	35.4	38.2	43.4	35.4	32.0	37.4	30.9–47.2	36.60
$CaBr_2$	50.5	41.1	40.0	37.9	42.4	41.3	45.1	46.3	48.7	42.3	38.3	45.3	37.9–50.5	43.27
$SrCl_2$	44.4	33.8	31.5	29.1	33.0	33.4	33.7	36.2	40.3	33.3	31.1	34.2	29.1–44.4	34.50
$BaCl_2$	39.3	26.5	25.6	30.0	32.5	33.0	35.5	38.7	40.3	33.5	31.4	34.9	25.6–40.3	33.42
$ZnSO_4$	21.1	15.6	14.1	13.9	15.6	15.8	17.2	18.5	20.7	15.9	15.2	16.9	13.9–21.1	16.71
$Al_2(SO_4)_3$	23.0	14.7	14.8	14.2	17.3	16.0	17.5	18.5	20.3	15.3	15.1	16.1	14.2–23.0	16.90
Average of salts	37.71	26.51	25.66	23.45	26.41	27.39	29.81	31.24	34.11	27.82	25.62	28.63		

TABLE V

PERCENTAGE OF TOTAL PROTEIN EXTRACTED FROM WHEAT FLOURS BY 2.0 NORMAL SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
LiCl	36.1	23.7	24.0	22.2	24.4	25.3	31.7	33.1	34.8	29.2	29.0	29.7	22.2–36.1	28.60
NaCl	27.5	17.4	16.1	16.1	16.1	17.7	19.1	21.5	22.4	17.6	15.7	20.5	16.1–27.5	18.97
Na_2SO_4	27.5	14.4	13.2	12.2	14.4	15.5	19.9	20.5	22.1	18.0	13.5	16.4	12.2–27.5	17.30
Na_3Citrate	45.3	17.4	16.0	14.3	15.5	19.7	32.6	33.9	35.7	21.7	25.2	16.9	14.3–45.3	24.52
Na_2HPO_4	43.0	18.2	18.3	14.1	15.6	20.3	34.4	34.0	37.2	22.3	26.2	20.2	14.1–43.0	25.32
KF	17.0	9.7	10.2	10.7	9.8	10.4	15.1	14.8	13.4	11.6	9.3	11.2	9.3–17.0	11.93
KCl	29.2	19.3	16.5	15.6	17.0	19.0	19.8	21.7	24.8	19.1	16.9	21.2	15.6–29.2	20.01
KBr	51.4	35.3	31.4	29.4	32.3	37.0	32.1	35.5	41.9	30.2	27.4	35.0	27.4–50.9	34.91
$MgCl_2$	51.1	36.2	36.0	31.3	34.8	35.2	38.3	41.9	46.0	37.2	32.8	39.4	31.3–51.0	38.35
$MgBr_2$	56.8	56.4	52.0	46.0	51.9	51.7	55.9	57.4	61.2	53.2	50.5	59.5	46.0–56.8	54.38
$MgSO_4$	40.8	19.9	21.4	17.9	19.7	22.9	28.8	31.2	33.5	25.2	24.7	22.3	17.9–40.8	25.69
$CaCl_2$	41.6	26.7	28.8	27.9	26.5	31.2	35.6	35.7	39.1	32.1	25.7	36.1	25.7–41.6	32.25
$CaBr_2$	59.6	49.1	56.2	48.7	49.7	55.3	54.9	56.7	58.7	53.7	46.5	55.1	46.5–59.6	53.68
$SrCl_2$	47.2	34.7	35.6	29.5	34.1	34.0	36.6	39.2	41.5	36.0	32.7	36.5	29.5–47.2	36.47
$BaCl_2$	26.9	25.5	27.3	26.6	28.0	28.1	31.4	32.3	34.3	32.3	27.1	31.1	25.5–34.3	29.24
Average of salts	40.07	26.93	26.87	24.17	25.99	28.22	32.41	33.96	36.44	29.29	26.88	30.07		

TABLE VI

AVERAGE PERCENTAGE OF TOTAL PROTEIN* EXTRACTED FROM 12 WHEAT FLOURS BY VARIOUS CONCENTRATIONS OF SALT SOLUTIONS

Salt	Concentration of salt solution			
	5% or 10%	0.5 N	1.0 N	2.0 N
Li Acetate	23.23	25.01	22.32	.....
LiCl	28.84	30.08	29.32	28.60
NaCl	19.20	23.16	21.68	18.97
Na_2SO_4	18.75	20.00	18.20	17.30
Na_3 Citrate	25.18	25.07	25.13	24.52
Na_2HPO_4†	22.85	27.44	25.62	25.32
KF	13.08	.....	13.07	11.93
KCl	20.56	24.62	22.77	20.01
KBr	38.80	38.77	37.22	34.91
KI	.....	.....	63.89	.....
K_2SO_4	18.12	19.68	18.59	.....
K_2 Tartrate	23.87	26.35	24.12	.....
$KCrO_4$	27.85	26.92	.....	.....
$MgCl_2$	34.77	33.01	34.99	38.35
$MgBr_2$	31.21	30.17	40.11	54.38
$MgSO_4$	25.15	26.18	26.35	25.69
$CaCl_2$	38.30	34.14	36.60	32.25
$CaBr_2$	33.97	33.90	43.27	53.68
$SrCl_2$	31.30	32.59	34.50	36.47
$BaCl_2$	26.64	27.29	33.42	29.24
$ZnSO_4$	.....	.....	16.71	.....
$Al_2(SO_4)_3$	.....	.....	16.90	.....

*Each figure represents the average of at least 24 separate nitrogen determinations.
†Used as *di valent*.

TABLE VII

PERCENTAGE OF NON-GLUTEN PROTEIN EXTRACTED BY 5% OR 10% SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
Li Acetate	175	109	108	103	111	122	143	138	139	122	94	111	94–175	122.9
LiCl	205	149	125	137	153	138	183	158	173	147	115	153	115–205	153.0
NaCl	138	96	87	95	92	95	109	104	111	101	78	112	78–138	101.5
Na_2SO_4	137	96	90	87	93	92	108	90	123	101	74	103	74–137	99.5
Na_3 Citrate	226	116	105	96	120	114	159	149	164	123	111	113	96–226	133.0
Na_2HPO_4	184	112	95	91	107	101	152	151	162	122	116	121	91–184	126.2
KF	98	63	62	59	65	61	77	74	77	69	52	73	52–98	69.2
KCl	144	105	93	97	106	102	122	113	125	108	80	114	80–144	109.1
KBr	274	235	200	178	213	196	215	195	216	183	152	210	152–274	205.6
K_2SO_4	123	93	85	91	92	89	112	98	105	96	70	100	70–123	96.2
K_2 Tartrate	175	122	111	107	125	112	145	132	140	128	99	124	99–175	126.7
K_2CrO_4	215	150	126	93	148	113	160	152	187	151	118	152	93–215	147.1
$MgCl_2$	238	175	191	168	171	180	197	180	189	201	145	179	145–238	184.5
$MgBr_2$	205	164	155	146	154	145	196	173	185	163	133	167	133–205	165.5
$MgSO_4$	195	122	118	110	121	120	154	142	152	129	109	126	109–195	133.2
$CaCl_2$	276	211	192	182	204	184	220	207	205	199	157	205	157–276	203.5
$CaBr_2$	213	172	156	158	178	168	215	180	193	182	149	174	149–215	178.2
$SrCl_2$	218	171	158	156	165	151	184	156	178	162	130	167	130–218	166.3
$BaCl_2$	173	143	133	133	145	128	161	143	147	142	116	134	116–173	141.5
Average of salts	190.1	137.1	125.8	120.4	134.9	126.9	158.5	143.9	156.4	138.4	110.4	138.8		

TABLE VIII

PERCENTAGE OF NON-GLUTEN PROTEIN EXTRACTED BY 0.5 NORMAL SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
Li Acetate	189	127	112	108	128	124	154	144	154	130	103	116	103–189	132.4
LiCl	203	171	147	144	154	144	178	161	178	154	129	153	129–203	159.7
NaCl	154	126	111	112	116	116	134	129	134	117	96	128	96–154	122.7
Na_2SO_4	145	102	96	91	100	98	123	107	118	108	77	110	77–145	106.2
Na_3 Citrate	216	122	100	98	117	118	169	153	139	129	117	116	98–216	132.8
Na_2HPO_4	193	122	101	107	123	124	199	176	187	149	130	128	101–199	144.9
KCl	164	135	119	119	128	120	144	130	143	128	99	140	99–164	130.7
KBr	266	235	206	187	217	192	219	198	202	184	155	214	155–266	206.0
K_2SO_4	140	101	99	94	100	97	112	107	112	106	77	110	77–140	104.6
K_2 Tartrate	189	129	132	111	135	134	171	150	145	132	122	126	111–189	139.7
K_2CrO_4	221	152	115	99	131	120	161	151	171	136	109	145	99–221	142.6
$MgCl_2$	228	178	172	159	179	154	197	175	181	167	138	174	138–228	175.2
$MgBr_2$	199	158	157	151	160	151	199	171	181	149	138	107	107–199	160.1
$MgSO_4$	193	131	135	118	135	128	163	139	159	133	110	123	110–193	138.9
$CaCl_2$	241	196	174	165	179	164	199	174	189	173	142	180	142–241	181.3
$CaBr_2$	213	172	156	158	178	168	215	180	193	182	149	174	149–215	178.2
$SrCl_2$	241	181	169	158	177	158	185	169	181	157	132	170	132–241	173.2
$BaCl_2$	191	138	136	126	138	129	165	143	159	150	117	146	117–191	144.8
Average of salts	199.2	148.7	135.4	127.9	144.2	135.5	171.5	153.2	162.6	143.6	118.9	142.2		

TABLE IX

PERCENTAGE OF NON-GLUTEN PROTEIN EXTRACTED BY 1.0 NORMAL SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
Li Acetate	155	108	103	98	112	110	139	129	140	115	95	115	95–155	118.2
LiCl	206	151	150	139	156	149	175	150	163	150	124	155	124–206	155.7
NaCl	134	119	110	111	106	109	130	118	129	115	85	118	85–134	115.3
Na_2SO_4	135	93	87	86	90	88	106	94	112	100	70	98	70–135	96.6
Na_3 Citrate	223	Lost	94	90	105	106	173	155	164	121	112	108	90–223	131.9
Na_2HPO_4	218	124	106	96	112	110	175	156	175	124	113	114	96–218	135.2
KF	95	67	61	61	66	64	72	71	74	69	51	79	51–95	69.2
KCl	160	122	112	108	120	113	131	124	133	121	84	122	84–160	120.8
KBr	256	221	195	175	209	182	213	190	198	187	150	201	150–256	198.1
KI	389	391	330	321	370	339	361	313	327	339	266	336	266–391	340.2
K_2SO_4	138	92	88	86	91	88	110	100	112	106	70	102	70–138	98.6
K_2 Tartrate	182	122	118	107	117	114	168	140	120	128	111	110	107–182	128.1
$MgCl_2$	247	193	180	169	181	172	204	181	193	176	146	188	146–247	185.8
$MgBr_2$	244	230	209	191	224	195	250	211	223	200	175	205	175–250	213.1
$MgSO_4$	219	149	120	113	123	122	160	150	151	127	115	126	113–219	139.6
$CaCl_2$	248	206	186	180	204	169	208	190	209	189	151	196	151–248	194.7
$CaBr_2$	265	231	218	220	244	211	264	230	234	226	181	236	181–265	230.0
$SrCl_2$	234	190	172	169	190	171	198	180	194	178	146	179	146–234	183.4
$BaCl_2$	207	149	140	175	187	169	208	193	193	179	148	182	140–208	177.5
$ZnSO_4$	111	88	77	81	90	81	101	92	100	85	71	88	71–111	88.7
$Al_2(SO_4)_3$	121	82	80	82	100	82	103	92	98	82	71	84	71–121	89.7
Average of salts	199.4	156.4	139.8	136.1	152.2	140.2	173.8	155.2	163.9	148.4	120.7	149.6		

TABLE X

PERCENTAGE OF NON-GLUTEN PROTEIN EXTRACTED BY 2.0 NORMAL SALT SOLUTIONS

Salt	Flour No.												Range of flours	Average of flours
	1	2	3	4	5	6	7	8	13	14	15	17		
LiCl	190	133	130	129	140	129	186	164	167	156	137	155	129–190	151.3
NaCl	145	98	88	94	93	90	112	107	108	94	74	107	74–145	100.8
Na_2SO_4	145	81	72	71	83	79	117	102	106	96	63	85	63–145	91.7
Na_3 Citrate	238	98	87	83	90	101	191	168	171	116	119	88	83–238	129.1
Na_2HPO_4	226	102	100	82	90	104	202	169	179	119	123	106	82–226	133.5
KF	89	55	56	62	56	53	88	73	64	62	44	59	44–89	63.4
KCl	154	108	90	91	98	98	116	108	119	102	80	111	80–154	106.2
KBr	270	198	171	170	186	190	188	176	201	161	129	183	129–270	185.2
$MgCl_2$	269	203	196	181	200	180	224	208	221	199	154	206	154–269	203.4
$MgBr_2$	298	317	283	267	299	264	328	285	294	284	238	311	238–328	289.0
$MgSO_4$	215	112	117	104	114	117	169	155	161	135	116	116	104–215	135.9
$CaCl_2$	218	150	157	162	152	160	209	177	188	172	121	189	121–218	171.2
$CaBr_2$	314	276	306	282	287	283	322	282	282	287	219	288	219–322	285.7
$SrCl_2$	248	195	194	171	196	174	214	195	200	192	154	191	154–248	193.7
$BaCl_2$	142	143	149	153	161	144	184	160	165	172	128	162	128–184	155.2
Average of salts	210.7	151.3	146.4	140.1	149.7	144.4	190.0	168.6	175.1	156.5	126.6	157.1		

RELATION OF HYDROGEN-ION CONCENTRATION AND BUFFER VALUE TO THE BAKING QUALITY OF FLOUR, PART I

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Introduction and Review of Earlier Work

It is widely believed in the milling trade that the more highly buffered a flour is the longer fermentation will it stand before the dough becomes properly ripe and fit for baking. Moreover, it has been said that a highly buffered flour not only takes longer to ripen in the dough stage but will remain ripe for a longer period before deterioration sets in.

These conclusions are possibly based on the well-known facts that a bakers' grade or clear flour is more resistant to fermentation and will withstand more drastic treatment in the bakehouse than the corresponding patent flour, and is at the same time more highly buffered. (It does not follow that a bakers' grade of one flour is necessarily stronger than a patent of any other flour; the conclusion applies only to the higher and lower divides of one and the same flour. A baker's grade all-English, for example, will not stand so long a fermentation as a patent Manitoba, although it may be more highly buffered.) These two characters vary together and, in consequence, a causal connection between them has been inferred. The experimental evidence for this conclusion is slender. The inference might be sound if these two variables were the only two differentiating bakers' grades from patents. Many other, possibly more important, variables are known to occur. Bakers' grades contain a larger proportion of flour from the outer layers of the endosperm than do patents; they therefore contain more ash, including phosphates (hence the increased buffer value), and more protein. Moreover, the gluten of bakers' grades is not only greater in amount but often different in quality; with the increased extraction, for example, some of the protein from the aleurone layer (which is sometimes regarded as the outer layer of the endosperm, and again as the inner layer of the six-fold skin of the wheat berry) may pass into the flour and this protein is not necessarily the same in quality or in chemical character as the

gluten of the rest of the endosperm. It is entirely possible that the increased strength and the increased buffer value may be consequences of the circumstances of production of the flour without there being any direct causal relation between the two. The problem is of fundamental importance and has not so far been adequately investigated.

The most important worker in this field has been Jessen-Hansen (1911) who, working in the Carlsberg laboratories, attempted to show that there was a particular H^+-ion concentration, or pH value, at which every flour made its best and largest loaf. Speaking very roughly, this pH varied somewhat from flour to flour and appeared to depend also to some extent on the nature of the acid that was added to the flour in order to alter the pH. Jessen-Hansen's conclusions are summarized in Table I.

TABLE I

SUMMARY OF JESSEN-HANSEN'S RESULTS

Original H^+-ion concentration of flour	H^+-ion concentration at which best loaf was made	Acid used
pH	pH	
A - 70% flours		
6.40	4.70	HCl
6.35	4.90	HCl
6.30	5.15	HCl
6.20	5.50	HCl
6.45	5.80	HCl
6.46	5.85	HCl
B - 30-50% patent		
6.10	4.44	HCl
6.25	4.60	HCl
6.10	4.65	HCl
	4.75	H_3PO_4
6.10	4.85	HCl
5.90	4.75	H_3PO_4
6.10	4.85	HCl
6.05	5.05	lactic
	5.20	H_3PO_4
C - 30-50% bottom		
6.20	4.55	lactic
	4.95	HCl
	5.20	H_3PO_4
6.10	4.85	acetic
6.35	5.05	HCl

Altogether Jessen-Hansen carried out twenty series of determinations on fifteen different flours, including patents, straight run, and low grade. The acid used for changing the pH of the flour was generally hydrochloric acid, although some experiments were carried out with lactic, acetic, and phosphoric acids. The H^+-ion concentration at which the largest and most satisfactory loaf was produced varied with different flours from pH = 5.85 to

4.44, the latter representing a H^+-ion concentration almost exactly 25 times the former. Jessen-Hansen concluded from these experiments that the optimum H^+-ion concentration, i. e., the pH at which the production of bread will be most successful, is approximately the same for all flours, being a little less than 5 for patent flours and a little greater for medium and low grade. There has been an unfortunate tendency among cereal workers to accept these conclusions of Jessen-Hansen somewhat uncritically as well-established scientific fact, rather than as purely provisional conclusions based on experiments with a large margin of error; the statement that pH 5 is the best H^+-ion concentration for bread making has found its way into the literature as a presumed fact and very little experimental work has been done on the subject since 1911.

Cohn and Henderson (1918) go so far as to say "the acidity of the dough at the time of baking seems to be the most important variable factor in bread making. Whilst 'proving' proceeds acids are formed which are helpful in making good bread." In view of the work described below neither of these statements can be regarded as well established on a sound experimental basis.

The work of Dunlap (1926), which appeared to support the conclusions of Jessen-Hansen, is vitiated by the fact that this worker altered the H^+-ion concentration of his flours by means of Beta Chlora, which is a mixture of approximately 99.5% chlorine and 0.5% nitrosyl chloride. This gas is well known to and widely used by English millers as a flour improver, especially for low-grade flours. It is one of the most effective chemical flour improvers in common use in England, but there is no evidence to show that the improvement brought about by chlorine is in any way connected with the accompanying alteration in H^+-ion concentration of the flour. On the contrary there is some evidence against this explanation: Very similar and certainly not less improvement may be brought about by Agene, the active principle of which is nitrogen trichloride, and in this case no detectable alteration in flour or dough H^+-ion concentration occurs. Moreover an equivalent amount of hydrochloric acid does not have an improving action. The work of Dunlap would have been less open to criticism if other acid substances such as hydrochloric or sulphuric acid, not known to have improving qualities, had been employed.

It may be well to point out that Jessen-Hansen was much less definite in his statements than many more recent workers, and concludes his paper as follows: "These experiments, which I have

just described, constitute fundamentally only one part of a more extended piece of work, of which the goal is to determine the correlation existing between the chemical composition of a flour and its baking value. However, as this work is still far from finished and as, nevertheless, the experiments described above appear to me to result in conclusions of some interest, I believe that there should be no longer a delay in their publication, although I know better than anyone that up to this point the experiments present a purely provisional character."

Not all workers, however, have accepted Jessen-Hansen's provisional conclusion as well ascertained fact. Bailey states (1925, p. 282) "While Jessen-Hansen's contention that the best bread resulted when dough acquired a H^+-ion concentration equivalent to $pH = 5.0 \pm$ may be acceptable, it appears probable that if this is a fact, it is because the increased acidity accelerates the activity of the enzymes of the dough, and particularly of diastase and zymase. *There is as yet no tangible evidence to prove that acidulation of the dough improves its physical properties in a direction that makes for better bread.*" (The italics are ours.) And again (Bailey, 1925, pp. 192-193) "that the observed progressive increase in hydrogen-ion concentration is the only factor responsible for the improvement in the baking strength of flour on aging appears to the author to be improbable. If acidity were the cause of improvement it would indeed be fortunate, since in that event all the advantages of natural aging, with perhaps the exception of bleaching, could be achieved by a regulated addition of hydrogen ions. Extended efforts to accomplish this effect by means of graduated additions of various acids have failed to yield the results of natural aging. Other changes seem to be in progress in stored flour, which, however, have thus far eluded detection and measurement."

Jessen-Hansen's technique was good for the time at which he worked (1910-11), but a large margin of error must inevitably occur in experiments the results of which depend on the making of a loaf. In each experiment four loaves were made from each batch of dough, and each experiment was repeated three or more times on different days; each figure for loaf volume given in Jessen-Hansen's tables is consequently the mean for at least twelve loaves. This procedure would decrease the error considerably, but he does not record what differences were observed between the individual loaves of a batch. The mean loaf volume of each replicate batch of four loaves varied, in the few data recorded in the paper, by

as much as 10%; the variation between individual loaves would be expected to be considerably greater than this. In view of such sources of error one is not justified in accepting Jessen-Hansen's results in any other way than that in which the author expected them to be accepted, *viz.*, as purely preliminary and provisional. The problem, however, is of such fundamental importance that it is surprising that after sixteen years the work still awaits confirmation.

In addition to the source of error discussed above, a further serious criticism can be brought against the work, although so far it appears to have escaped notice. A single invariable fermentation period was employed throughout the investigation except for the last, or proof period, which was varied with the different types of flours. The influence of H^+-ion concentration (or of any other factor) on loaf quality cannot be adequately investigated by employing a fixed fermentation time. The effect of acidity on loaf quality may be indirect and complex to a greater degree than it is direct and simple. Length of fermentation is one of the most important factors affecting loaf quality, and this, too, may be affected by H^+-ion concentration; the length of fermentation that may yield the best loaf at one H^+-ion concentration may be longer or shorter than that which will yield the best loaf at another. The most suitable H^+-ion concentration should therefore be considered the most suitable only when the fermentation period is also the most suitable; otherwise no strict comparison can be made. This is an important generalization, the fundamental character and truth of which will be more fully illustrated by the experimental work described below. It is entirely possible that, had Jessen-Hansen varied his fermentation times so as to ensure that each loaf was made at the correct time for maximum quality, his generalization regarding the optimum H^+-ion concentration for bread making might have been expressed very differently.

Experimental Results

In view of the large amount of experimental work that is being carried out in the writers' laboratories in connection with problems of panary fermentation, it has been considered desirable in the first instance to repeat Jessen-Hansen's work under more rigidly controlled conditions, and to study one or two flours intensively before extending the observations to a larger number of flours.

Two flours were chosen for investigation:

(A) A fine patent flour well known to most English bakers, and

(B) A straight run (72% extraction) imported Canadian flour, manufactured from a blend of graded Manitoba wheats, probably a mixture of No. 1 and No. 3 Manitoba.

A, as a fine patent, is likely to be more sensitive to outside influences, such as added acids or prolonged fermentation, than flours of longer extraction. B, being of longer extraction than A, would not be unduly sensitive but should, on the contrary, stand more drastic treatment than A.

The analytical results and gas production figures for these two flours are given in Tables II and III.

TABLE II

ANALYTICAL RESULTS FOR FLOURS A AND B

	Patent flour A	Straight run flour B
	per cent	per cent
Moisture	15.56	14.91
Ash	0.36	0.48
Total nitrogen (N)	1.71	2.06
Soluble extract	5.22	5.33
Soluble nitrogen	0.424	0.236
Soluble phosphate (P_2O_5)	0.040	0.085
Total phosphate (P_2O_5)	0.157	0.208
Total acidity	0.43	0.33
Crude protein or gluten (N x 5.7)	9.75	11.74
pH (of flour suspension)*	5.71	6.16
Buffer value (of flour suspension)*	0.66	0.64

*The H^+-ion concentration and buffer values were determined on decantates obtained from the suspensions of flour in water and not on the filtered aqueous extracts. The H^+-ion concentration is not seriously affected by this change of procedure; the buffer value, however, is quite different from that of the filtered extract because the lactic acid is added to the suspension and not to the extract. The point of interest, after all, is the buffer value of the flour-water mixture, or the dough.

The patent flour will be discussed first.

Nineteen pounds of flour was made into a dough with 6 ounces (= 2% of the weight of flour) of yeast, 3¾ ounces (= 1¼% of the weight of flour) of salt, and sufficient water. The temperature of the thoroughly kneaded dough was 80°F., and this temperature was maintained throughout the whole fermentation period. The dough was placed in a proving cabinet (maintained at 80°F.) for fermentation to proceed. The first dough was scaled off after 1 hour, was allowed to prove for 35 to 40 minutes, and was placed in the oven exactly 1¾ hours after the dough was mixed. A further scaling and baking was carried out at ¾-hour intervals up to 11 hours. Two-pound tinned loaves were made in all cases.

TABLE III

GAS PRODUCTION OF FLOURS A AND B

(50 gm. flour, 1 gm. (= 2%) yeast, 0.625 gm. (= 1¼%) salt, 30 cc. (=67.2 qt. per sack) water, temperature 80° F.)

Time	Patent flour A		Straight run flour B	
	No acid added	+6 oz. tartaric acid per sack	No acid added	+6 oz. tartaric acid per sack
	cc.	cc.	cc.	cc.
1st hour	40	32	29	43
2nd hour	77	98	81	78
3rd hour	95	110	99	93
4th hour	101	103	94	127
5th hour	84	77	103	114
6th hour	55	47	86	92
7th hour	36	33	74	57
8th hour	24	28	60	33
Total gas in 24 hours	770	783	942	933

At the same time another dough was made up similar in all respects to the first, except that tartaric acid, 6 ounces per sack of flour, was dissolved in the liquor. This dough behaved similarly to the untreated dough at all stages of fermentation, but remained consistently tougher with slightly better stability. Doughs were scaled off and baked at ¾-hour intervals as with the former dough; corresponding loaves of the two series, i.e., one loaf with tartaric acid and one loaf without, were baked together. Both pH and buffer values were determined electrometrically, by means of the quinhydrone electrode, on the doughs at scaling time and (next day) on the loaves. These determinations were made, not on the filtered aqueous extracts, but on the decantates obtained from suspensions of dough or loaf crumb in water. The results are given in Table IV and Figure 1. Every dough was examined at scaling and proving and all loaf characters were noted and recorded. No attempt was made to classify the loaves according to a system of "bakers' marks" or other supposed quantitative system. Such systems are used by many workers, especially in America; their application is, however, difficult, much experience of loaf judging is required and the final mark generally conveys very little information to anyone except the individual worker using the system. Moreover, its supposed quantitative character is not really quantitative, as it depends ultimately on personal judgments of color, texture, etc., and consequently tends to afford a false sense of accuracy. Our own object was to pick out the best loaf of each series and to compare it with the best of the other series. Thus no attempt was made to *measure* loaf volume; by cutting the loaves transversely and symmetrically through the centers and placing halves of different loaves together it is easy to arrange the loaves in order of size. As the fermentation time increased, loaf volume

first increased to a maximum and then decreased. With the two series under review, the biggest loaf was also the best in all characters. In the series containing no added tartaric acid, the best and largest loaf was 'thrown' at 3¼ hours; the two loaves produced at 2½ and 4 hours, respectively, were not unlike each other, although the one was slightly under- and the other slightly over-fermented; but both were inferior to the 3¼ hour loaf. In the series containing 6 ounces of tartaric acid per sack, the best loaf was the one produced at 2½ hours fermentation. The two series resembled each other remarkably closely, and it was distinctly difficult to distinguish the best loaf of one series from the best of the other, in spite of the fact that one loaf had a pH of 5.75 and a buffer value of 0.90, and the "acid" loaf had a pH of 4.93 and a buffer value of 0.52. The only considerable difference between the two loaves was in flavor (taste): the "acid" loaf had a more pronounced flavor than the "blank." We ourselves found the flavor distinctly pleasing, but it is possibly too definite to be generally popular.

TABLE IV

EFFECTS OF INITIAL H^+-ION CONCENTRATION AND LENGTH OF FERMENTATION ON THE FINAL H^+-ION CONCENTRATION AND BUFFER VALUE OF PATENT FLOUR DOUGHS AND LOAVES, FIRST SERIES

Time from making doughs	No tartaric acid added			6 oz. per sack of added tartaric acid		
	H^+-ion concentration as pH		Buffer value	H^+-ion concentration as pH		Buffer value
	of dough	of loaf	of loaf	of dough	of loaf	of loaf
hr.						
0	5.74	.....	.....	4.94	.....	.....
1	5.62	.....	.....	4.88	.....	.....
1¾	5.57	5.81	0.90	4.87	4.99	0.59
2½	5.46	5.78	.93	4.90	4.93	.52
3¼	5.52	5.75	.90	4.88	5.15; 5.07	.71
4	5.46	5.70	.88	4.87	4.90	.50
4¾	5.50	5.65	.86	4.95	4.93; 4.95	.53
5½	5.41	5.62; 5.44	.88	4.87	4.92; 4.85	.54
6¾	5.38	5.58	.85	4.99	4.89	.50
7¾	5.29	5.58; 5.46	.92	4.79	4.86	.52
8½	5.29	5.56	.85	4.85	4.89	.60
9¼	5.28	5.53	.86	4.95	4.98; 4.99	.58
10	5.30	5.50	.95	4.85	4.87	.57
11	.....	5.52; 5.30	0.91	.....	5.09; 5.15	0.66

An examination of Table IV and Figure 1 shows that, while in each series the pH of dough and loaf showed a general tendency to fall (i. e., the H^+-ion concentration increased) as fermentation proceeded, considerable irregularity occurred, especially in the acid series. Also, of the few duplicate determinations carried out, some showed good agreement and some did not. These irregularities were attributed (a) to insufficient mixing of the ingredients during the kneading of the dough, (b) to the small size of the dough and loaf samples taken

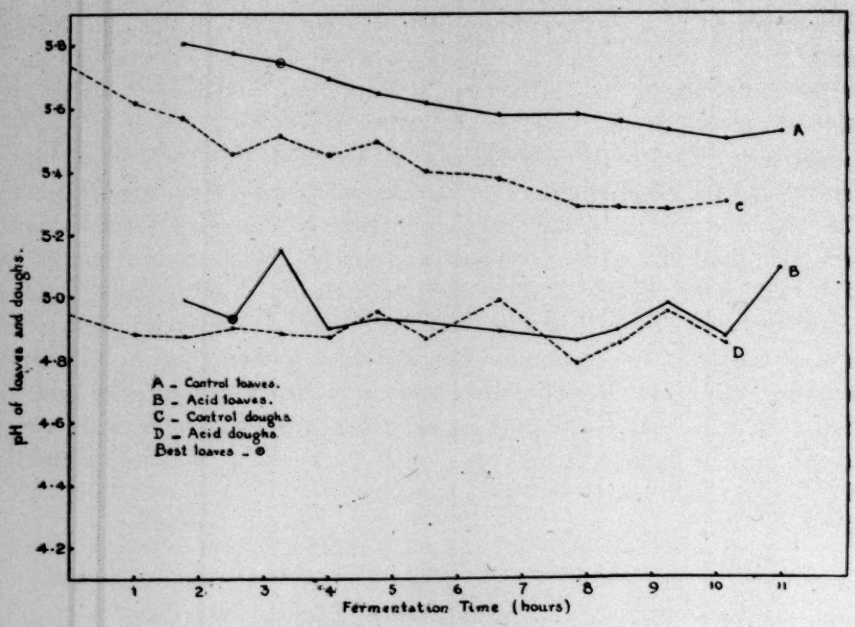

Fig. 1. Patent Flour

for the pH determinations. The whole acid series was therefore repeated with the following modifications in technique. Instead of one twelve-loaf batch of dough being made at one time, four three-loaf batches were made with thorough mixing. These were then incorporated into one batch which was given a final and very thorough kneading. At each scaling time four small portions were taken from the interior of the dough and pH determinations carried out on them. In spite of the very thorough kneading it was found impossible to obtain concordant replicate pH readings on small portions of dough (see Table V.) Evidently thorough mixing from a baking point of view is something quite different from complete and uniform incorporation from a scientific point of view. On account of the nature of dough it appears to be impossible (unless the operation is carried on to such an extent that, for baking purposes, the dough is ruined) by any system of hand-kneading or perhaps of machine-kneading to make a dough as uniform in its composition as, say, an aqueous solution of mixed salts. It is possible to get excellent agreement in replicate determinations of the pH of *flour;* it is not possible to rely on getting good agreement in replicate determinations on *dough,* and it is impracticable to use very large samples. The difficulty was overcome in the case of the loaves by scooping out 100 grams of the crumb from one half-loaf for the

determination of the pH value. This crumb was thoroughly mixed with 350 cc. distilled water and the pH determined on the supernatant liquid after standing and settling for half an hour. One hundred grams of the crumb of the second half of the loaf was mixed with 100 cc. N/ 100 lactic acid and 250 cc. water and the pH of the supernatant liquid was determined after half an hour; the difference of the two pH values was regarded as the buffer value, and this would be numerically smaller the greater the buffering effect. The results of this series are given in Table V, and, together with those of the former "blank" series, in Figure 2 and the dotted curve of Figure 4.

TABLE V

VARIATIONS IN H^+-ION AND BUFFER VALUES DURING FERMENTATION OF PATENT FLOUR DOUGH AND LOAVES

Second Series, Containing 6 oz. Tartaric Acid per Sack

Time from making dough	pH of dough	pH of loaf	Buffer values of loaf
hr.			
0	4.90, 5.00, 4.97, 4.94	....	....
1	4.92, 4.85, 4.80, 4.84	....	....
1¾	4.91, 4.89, 4.95, 4.92	5.06	0.61
2½	- - - -	5.04	.61
3¼	4.89, 4.89, 4.87, 4.89	5.02	.61
4	4.87, 4.88, 4.86, 5.02	5.00	.60
4¾	4.78, 4.96, 4.91, 4.83	4.98	.59
5½	4.79	4.96	.59
6¼	4.82, 4.79, 4.72, 4.72	4.95	.58
7	4.81, 4.78, 5.01, 4.79	4.93	.56
7¾	4.95, 4.77, 4.79, 4.74	4.91	.54
8½	4.74, 4.72, 4.73, 4.77	4.89	.53
9¼	- - - -	4.90	0.53

In the untreated series the H^+-ion concentration of the loaf in terms of pH changed from 5.75 (at 3¾ hours fermentation) to 5.50 (at 10 hours fermentation); this difference of only 0.25 in pH is surprisingly small in view of the character of the loaf produced by 10 hours fermentation. This loaf was small, the crumb was dark, harsh, tough, and contained large holes. It possessed a strong sour smell. Very evidently the sourness produced by long fermentation may be due to acid production but cannot possibly be due to production of H^+-ions. This conclusion is amply confirmed by the fact that by adding sufficient acid to the dough at the start to change the pH, not by 0.25 but by 0.82, no effect at all (other than one of flavor) was produced on loaf quality, while surprisingly little effect was produced on dough character; the dough was somewhat tougher and became ripe and produced the best loaf ¾ hour earlier than that of the untreated flour.

The data given in Tables IV and V and shown graphically in Figure 2 are of great interest and will repay careful study. In every case the H^+-ion concentration of the loaf was greater than that of the

corresponding dough in the "blank" series by about pH = 0.20 to 0.30 and in the "acid" series by approximately pH = 0.15; that is, the actual baking raises the pH or diminishes the H^+-ion concentration, considerably. Again, between 1¾ and 10¼ hours fermentation a steady and regular fall in pH (or rise in H^+-ion concentration) of the loaf occurred, a fall of 0.31 in the "blank" series and of 0.16 in the "acid" series; this smaller fall corrresponds to the fact that the added tartaric acid nearly doubled the buffer value (cf. Table IV). The last column in Table V and the dotted curve in Figure 4 show the very slight but steady and progressive rise in buffer value as fermentation proceeds.

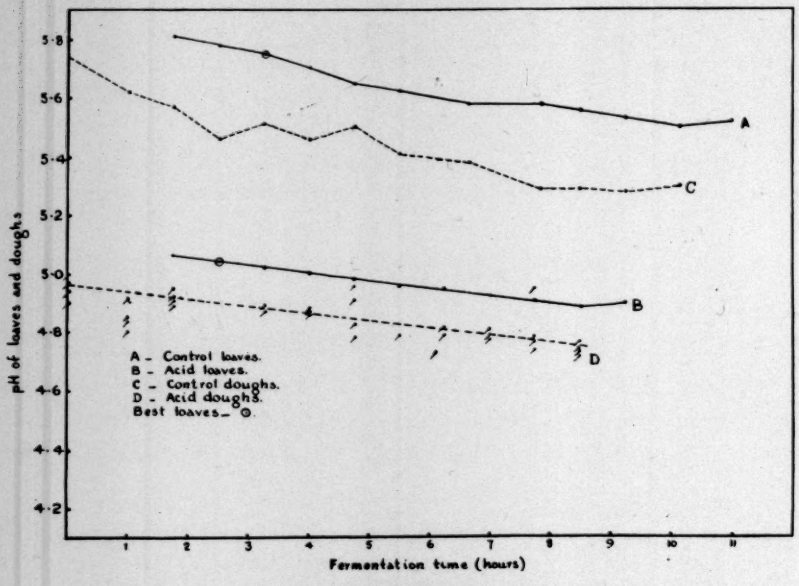

Fig. 2. Patent Flour

The same method of investigation was applied to the straight run flour. As with the patent flour the acid dough was tougher than the untreated; in all other respects the two doughs were alike. Both loaf volume and loaf quality (in all characters) increased to a maximum and then decreased as fermentation proceeded. The best and largest loaves from both doughs were produced after 3¼ hours fermentation (including the proving period). These two loaves were of excellent quality in all respects; they were virtually duplicates and so much alike that an experienced baker would have difficulty in distinguishing them, the only obvious difference being one of flavor (taste). The loaves produced at 2½ and 4 hours fermentation were quite good and

much alike, but distinctly inferior to the 3¼-hour loaves. With this flour the added tartaric acid, although it lowered the pH of the best loaf from 6.07 to 5.20, i.e., by 0.87 (as against 0.71 with the patent flour), had no effect on fermentation time; from a baking point of view the two series of loaves were essentially duplicates.

The pH and buffer values of the straight run doughs and loaves are given in Table VI and Figures 3 and 4. Single pH determinations were carried out on the doughs; these were a little irregular, but show on the whole a small decrease with increase in fermentation time. The pH of the loaves in the untreated series showed a steady decrease as fermentation proceeded; in the "acid" series the pH decreased from 5.31 at 1¾ hours to 5.10 at 6¼ hours fermentation, after which it remained unchanged at 5.10 within 0.01, a very small quantity and undoubtedly within the experimental error of the method of work. In the "blank" series the pH dropped during the whole fermentation period about 0.45, compared with 0.20 for the "acid" series; this difference, as with the patent flour series, is connected with the increased buffer value due to the added tartaric acid.

TABLE VI

EFFECTS OF ORIGINAL H^+-ION CONCENTRATION AND LENGTH OF FERMENTATION ON THE FINAL pH AND BUFFER VALUE OF STRAIGHT RUN FLOUR DOUGHS AND LOAVES

Time from making dough	No tartaric acid added			6 oz. per sack of tartaric acid added		
	pH		Buffer value	pH		Buffer value
	of dough	of loaf	of loaf	of dough	of loaf	of loaf
hr.						
0	5.62	.....	.....	5.10	.....	.....
1	5.64	.....	.....	5.22	.....	.....
1¾	5.59	6.12	0.84	5.11	5.31	0.66
2½	5.64	6.01	.76	5.31	5.26	.60
3¼	5.59	6.07	.83	5.05	5.20	.60
4	5.57	5.99	.80	5.16	5.18	.59
4¾	5.53	5.92	.75	5.02	5.16	.55
5½	5.47	5.86	.75	4.95	5.14	.53
6¼	5.50	5.86	.76	4.98	5.01	.54
7	5.47	5.82	.73	4.99	5.10	.50
7¾	5.61	5.82	.74	5.05	5.11	.54
8½	5.54	5.77	.69	5.07	5.09	.53
9¼	5.50	5.79	.68	5.09	5.11	.55
10	5.45	5.75	.69	4.93	5.09	.53
10¾	5.49	5.73	.64	4.99	5.10	.52
11½	5.48	5.69	.64	5.06	5.11	.53
12½	5.42	5.70	.64	4.92	5.10	.52
13	.....	5.66	0.63	.....	5.09	0.52

An attempt was made to ascertain to what extent the increased buffer value was directly due to the presence of the tartaric acid, or whether it was due to increased soluble phosphate content brought into solution by the added acid. Loaves Nos. 1, 4, 8, 12, and 16 of the

"blank" series and loaves Nos. 1, 5, 9, 13, and 16 of the "acid" series were analyzed for soluble phosphate. The results are given in Table VII and indicate that the increased buffer value due to added tartaric acid is at any rate partly attributable to the additional soluble phosphate brought into solution by the tartaric acid.

TABLE VII

BUFFER VALUES AND SOLUBLE PHOSPHATE CONTENTS OF LOAVES AS AFFECTED BY PRESENCE OF TARTARIC ACID
(6 oz. per Sack)

Loaf No. in series	Buffer values		Soluble phosphate (P_2O_5) content	
	No added acid	+added acid	No added acid	+added acid
			per cent	per cent
1	0.84	0.66	0.068	0.091
4	.80	....	.081	.....
5	....	.55	.....	.093
8	.73	....	.079	.....
9	....	.54	....	.089
12	.69	....	.084	.....
13	....	.52	.....	.089
16	0.63	0.52	0.087	0.091

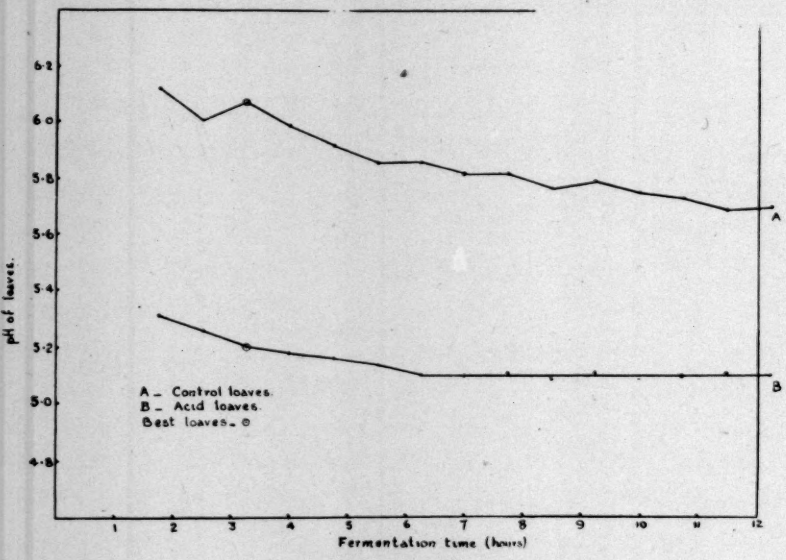

Fig. 3. Straight Run Flour

One possible criticism of the results given above is that the range of H^+-ion concentration dealt with (= pH = 0.80 to 0.90) is so great that the best loaf may have been bracketed by these extreme limits. For example, with the straight run flour the best loaf of the "blank" series had a pH of 6.07 and that of the "acid" series 5.20. It is possible that a better loaf would have been obtained at an intermediate

pH of 5.65; that is, loaf quality might be improved between pH 6.07 and 5.65 and fall off somewhat from 5.65 to 5.20. To meet this objection three series of loaves were baked from the straight run flour with a fermentation period of 3¼ hours (including final proof) with varying tartaric acid contents of 0, 2, 4, 6, and 8 ounces per sack. In these series again the doughs with 6 and 8 ounces per sack were tougher and showed slightly better stability than the "blank"; the differences, however, though distinct, were not really striking and were barely noticeable with the smaller quantities of acid, i.e., less than 6 ounces per sack. The pH and buffer values of one series are given in Table VIII. The loaves of all three series were closely similar, the only noticeable differences being (1) the crumb color of loaf 1 (containing no added acid) was not quite so good as that of the acid loaves; and (2) the volumes of loaves 4 and 5 were slightly less than those of loaves 1 to 3 in the first two series; little difference in volume was observable between any of the loaves of the third series. The loaves were baked (five at a time) in a 4-loaf electric oven, and under these conditions complete identity of volume could hardly be expected. On the whole, these experiments confirm the earlier results.

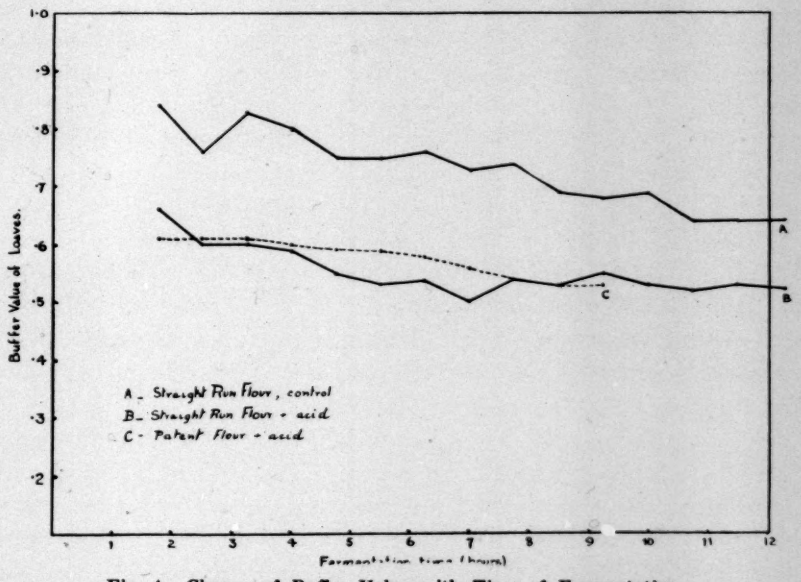

Fig. 4. Change of Buffer Value with Time of Fermentation

TABLE VIII

H$^+$-ION CONCENTRATION AND BUFFER VALUES OF LOAVES BAKED AFTER 3¼ HOURS FERMENTATION (2% YEAST, 1¼% SALT, AT 80° F.) AND CONTAINING VARYING AMOUNTS OF TARTARIC ACID

No. of loaf	Amount tartaric acid added, per sack	Area of loaf, cross-section	H$^+$-ion concentration of loaf, as pH	Buffer value of loaf
	oz.	sq. in.		
1	0	30	5.95	0.69
2	2	31	5.71	.65
3	4	29	5.52	.62
4	6	30	5.33	.57
5	8	27	5.16	0.54

Summary and Conclusions

The conclusions to be drawn from the experiments discussed appear to us unmistakable: with the two flours studied, H$^+$-ion concentration was a factor of little importance in bread making and contributed little or nothing to loaf quality. With the patent flour the fermentation was slightly hastened by the increased H$^+$-ion concentration of the dough, but this effect was not observed with the straight run flour. The sole unmistakable effects that we have noticed as directly due to increased H$^+$-ion concentration were on dough toughness and on loaf flavor (taste), and even these effects were only pronounced with large increases, e.g., a fall of 0.80 in pH. It is very evident that acid substances are produced during fermentation; after prolonged fermentation the smell of the dough or of the loaf is very "strong" and typically "acidic" in character. The observed ill effects on dough and loaf quality cannot, however, in the light of the experiments described above, be due either directly or indirectly to the small increase in H$^+$-ion concentration actually observed. Many other chemical and colloid changes go on in a fermenting dough and all of them doubtless contribute something to the final product. Many of these changes must be of far greater importance as regards their effects on dough and loaf quality than any small change of H$^+$-ion could ever be.

These conclusions have been definitely established for two flours only. The evidence, however, is so striking that the presumption appears not unreasonable that similar results will be found with other flours. Whether this assumption is true can be determined only by investigation. The point, moreover, is of such great interest and importance that the work is being extended to cover a wide range of flours and flour blends.

The results of these further investigations will be presented in a later communication.

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FLOUR COLOR TESTS

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Introduction

Kent-Jones and Herd (1927) drew attention to a numerical method for recording the color of flour in which two distinct estimations were made. They employed the usual gasoline method for the extraction of the carotin, thereby obtaining a numerical standard for the yellowness of the flour. They further employed alkaline methyl alcohol with the idea of extracting the coloring matter due to the presence of finely ground offal, thereby determining the brightness, or grade, of the flour. A consideration of both these figures permits not only a visualization of the general color effect but the establishment of a useful numerical record. They advocated the examination of 50 cc. of the extracts obtained in a specially devised colorimeter, which was described. This method has been criticized by Visser't Hooft and de Leeuw in a paper read before the American Association of Cereal Chemists, at Minneapolis (1928). They made an able criticism of the method and pointed out certain errors. In essence their criticism was as follows:

1. The principle of the Kent-Jones' colorimeter, which involves the use of different quantities of a standard solution, gives rise to error. This error is said to be due to differences in H^+-ion concentration of the matching solutions containing the varying amounts of the standard coloring solutions and can be largely corrected by the employment of buffer solutions instead of distilled water.
2. The extraction with alkaline methyl alcohol, in order to obtain the grade figure, is not complete in the 16 hours specified by Kent-Jones and Herd, and as long as 40 hours is necessary.

Visser't Hooft and de Leeuw agreed that the colorimeter used by Kent-Jones and Herd was less tiring to the eye than the Duboscq, and also that the alkaline methyl alcohol figure did give an indication of the bran content of the flour.

In the method advocated by Kent-Jones and Herd (1927), the matching of the 50 cc. of extract was done by running into water sufficient standard color solution so that the two matched when examined in the colorimeter, which had one eye-piece with a split field. Visser't Hooft and de Leeuw showed that values obtained by the Kent-Jones and Herd method were different from those by the ordinary method using the Duboscq colorimeter, if both results were expressed as the number of cubic centimeters of a standard color solution necessary to match one cc. of the extract. The strength of potassium chromate solution used in this calculation was 0.005 per cent and the figures obtained by the Kent-Jones and Herd method, where a stronger coloring solution was used, were reduced to this unit.

Experimental—Gasoline Value

The authors have confirmed, in general, the results obtained by Visser't Hooft and de Leeuw. Table I shows the carotin results obtained on a number of flours, as estimated by the number of cubic centimeters of 0.005 per cent potassium chromate solution necessary to match one cc. of 20 per cent flour extract, the estimation being carried out both by the Kent-Jones and Herd method and by the use of the Duboscq colorimeter. It will be seen, when both are judged by the same standard (columns II and III), that often the Kent-Jones and Herd figures are about twice as large as those obtained with the Duboscq colorimeter. Actually, the agreement is closer with the less highly colored flours.

TABLE I

COMPARISON OF RESULTS OBTAINED WITH THE KENT-JONES–HERD AND THE DUBOSCQ COLORIMETERS

Sample	I cc. used in Kent-Jones' method	II Gasoline value* with Kent-Jones colorimeter	III Gasoline value with Duboscq colorimeter
X 1	13.5	2.70	1.15
C 374	10.0	2.00	1.10
C 375	9.5	1.90	1.05
X 2	9.0	1.80	1.00
M 283	8.0	1.60	0.90
M 282	8.0	1.60	0.85
X 3	7.5	1.50	0.70
X 4	7.0	1.40	0.65
X 5	7.0	1.40	0.65
W/w 37	6.5	1.30	0.60
X 6	5.5	1.10	0.50
X 7	4.0	0.80	0.40
M 279	1.5	0.30	0.25

*The gasoline value is taken as the number of cubic centimeters of 0.005% potassium chromate solution required to match one cc. of extract, in this case, the Kent-Jones figures divided by 5.

In an attempt to explain the discrepancies found, Visser't Hooft and de Leeuw diluted certain gasoline extracts in definite ratios with gasoline. Actually, they took 10 cc. of a particular extract and made this up to 100 cc. with clear gasoline. Similarly, 25, 50, and 75 cc. of the extract were made up to 100 cc. In this way they obtained five solutions in which the coloring matter was in the ratio of 10 : 25 : 50 : 75 : 100. They said:

> "It could therefore be expected that the gasoline values would show the same ratio and that the gasoline value of the original liquid could be calculated from the others by multiplying by 10, 4, 2, and 4/3."

They took the gasoline values of these extracts in both the Kent-Jones and the Duboscq colorimeters. Their results are shown in Table II. From this they concluded that the method used in the Kent-Jones colorimeter was wrong.

Visser't Hooft and de Leeuw repeated this experiment using, instead of the color standard suggested by Kent-Jones and Herd, a standard suggested by Sprague (1928). This consists of adding 3.4 cc. of a ½ per cent aqueous solution of napthol yellow and 0.5 cc. of a ½ per cent aqueous solution of orange G crystals to one liter of distilled water. The tint of this standard is said to be identical with that of pure carotin contained in pure gasoline. The standard is equivalent to a carotin solution containing 8.90 mgm. When Visser't Hooft and de Leeuw used this color standard in their dilution experiments, the two colorimeters in question were

found to agree and they therefore concluded that the error in the Kent-Jones and Herd method was due to the inorganic solution employed.

TABLE II

COMPARISON OF RESULTS OBTAINED WITH KENT-JONES AND DUBOSCQ COLORIMETERS (VISSER' HOOFT AND DE LEEUW [1928])

Inorganic standard solution used, aqueous-cobalt-chromate

	Kent-Jones colorimeter			Duboscq colorimeter	
	cc. used	Gasoline color value	Gasoline color value, original extract	Gasoline color value	Gasoline color value, original extract
Original extract	11.9	2.38	2.38	1.31	1.31
75 cc. or. extr. 25 cc. gas.	8.2	1.64	2.20	0.99	1.32
50 cc. or. extr. 50 cc. gas.	4.8	0.96	1.92	0.65	1.30
25 cc. or. extr. 75 cc. gas.	2.0	0.40	1.60	0.32	1.28
10 cc. or. extr. 90 cc. gas.	0.7	0.14	1.40	0.13	1.30

The color solutions suggested by Kent-Jones and Herd were as follows:

1. Standard Solution for Alkaline Methyl Alcohol
   Five cc. of 0.5 per cent potassium chromate solution, and 2 cc. of 10 per cent anhydrous cobalt nitrate solution. This is made up to 100 cc. with distilled water.
2. Standard Solution for the Carotin Extract
   Ten cc. of 0.5 per cent potassium chromate solution, and 1.5 cc. of 10 per cent anhydrous cobalt nitrate solution. This is made up to 100 cc. with distilled water.

In view of these criticisms the authors undertook a definite investigation into the matter, and repeated, in the first instance, the dilution experiment recorded by Visser't Hooft and de Leeuw. Although this was repeated on numerous occasions, not only by the authors but by independent and outside operators, they were unable to confirm the results obtained by the Dutch investigators. Table III shows the results obtained, in the Kent-Jones and the Duboscq colorimeters, on three flours—A, B, and C. Column I gives the cubic centimeters of color standard recommended by

Kent-Jones and Herd required for 50 cc. of the extract; column II, the gasoline value of one cc. of the extract, reduced to the basis of the standard used in the Duboscq, that is, 0.005 per cent potassium chromate solution. Column III gives the gasoline value of the original extract as calculated from column II by multiplying by the ratio of dilution. Columns IV and V correspond to columns II and III, but are taken in the Duboscq colorimeter. It will be seen that, altho there is disagreement between the figures obtained in the Kent-Jones and in the Duboscq colorimeters, both give an approximately constant result with the dilution method.

TABLE III

COMPARISON OF RESULTS OBTAINED WITH KENT-JONES AND DUBOSCQ COLORIMETERS, USING GASOLINE SOLUTIONS OF INCREASING DILUTION

(Original aqueous cobalt-chromate standard)

	Kent-Jones colorimeter			Duboscq colorimeter	
	I cc. used	II Gasoline value	III Gasoline value original extract	IV Gasoline value	V Gasoline value original extract
			Flour A		
Original extract	12.8	2.56	2.56	1.45	1.45
75 cc. orig. extr. 25 cc. gas.	8.9	1.78	2.38	1.05	1.40
50 cc. orig. extr. 50 cc. gas.	6.2	1.24	2.48	0.70	1.40
25 cc. orig. extr. 75 cc. gas.	2.7	0.54	2.16	0.35	1.40
10 cc. orig. extr. 90 cc. gas.	1.0	0.20	2.00	0.20	2.00
			Flour B		
Original extract	7.4	1.48	1.48	0.87	0.87
75 cc. orig. extr. 25 cc. gas.	5.8	1.16	1.54	0.65	0.88
50 cc. orig. extr. 50 cc. gas.	3.5	0.70	1.40	0.42	0.84
25 cc. orig. extr. 75 cc. gas.	1.7	0.34	1.36	0.27	1.08
10 cc. orig. extr. 90 cc. gas.	0.7	0.14	1.40	0.14	1.40

TABLE III—*Continued*

TABLE III

COMPARISON OF RESULTS OBTAINED WITH KENT-JONES AND DUBOSCQ COLORIMETERS, USING GASOLINE SOLUTIONS OF INCREASING DILUTION
(Original aqueous cobalt-chromate standard)

	Kent-Jones colorimeter			Duboscq colorimeter	
	I cc. used	II Gasoline value	III Gasoline value original extract	IV Gasoline value	V Gasoline value original extract
			Flour C		
Original extract	10.5	2.10	2.10	1.35	1.35
75 cc. orig. extr. 25 cc. gas.	7.7	1.54	2.04	0.90	1.20
50 cc. orig. extr. 50 cc. gas.	5.1	1.02	2.04	0.70	1.40
25 cc. orig. extr. 75 cc. gas.	2.5	0.50	2.00	0.32	1.28
10 cc. orig. extr. 90 cc. gas.	1.0	0.20	2.00	0.13	1.30

The authors are totally unable to understand the results obtained by Visser't Hooft and de Leeuw. Visser't Hooft and de Leeuw were of the opinion that an explanation of their results could be obtained in the varying H^+-ion concentration of the standard solutions used for matching, in the Kent-Jones and Herd method. They found that the dilution method gave satisfactory results and that there was good agreement between the Kent-Jones colorimeter and the Duboscq if, instead of making up the solution with water (as recommended by Kent-Jones and Herd), a buffer solution was employed consisting of 95 parts primary potassium phosphate solution and 5 parts secondary sodium phosphate solution, both 1/15 molar concentration (Sørensen). This liquid has a pH of 5.6. Table IV shows results obtained by Visser't Hooft and de Leeuw using this method. It will be noted from this table that these investigators not only found that the calculated gasoline value of the original extract kept constant in this dilution method, but that there was satisfactory agreement between the two colorimeters.

TABLE IV

COMPARISON OF GASOLINE VALUES OBTAINED WITH AND WITHOUT BUFFER SOLUTION (VISSER'T HOOFT AND DE LEEUW 1928)

	Kent-Jones colorimeter				Duboscq colorimeter			
	Water		Buffer solution*		Water		Buffer solution*	
	Gasoline value	Gasoline value original extract	Gasoline value	Gasoline value original extract	Gasoline value	Gasoline value original extract	Gasoline value	Gasoline value original extract
Original extract	4.70	4.70	2.60	2.60	2.55	2.55	2.60	2.60
50 cc. orig. extr. 50 cc. gas.	2.20	4.40	1.40	2.80	1.21	2.42	1.30	2.60
75 cc. orig. extr. 25 cc. gas.	0.80	3.20	0.72	2.88	0.64	2.56	0.60	2.40

*Cobalt-chromate buffered at H^+-ion of pH=5.6.

The authors repeated this work using a number of gasoline extracts of flours at various dilutions. The results are given in Table V. It will be seen from this table that there is good agreement between the results obtained by the Kent-Jones colorimeter and the Duboscq, but that the agreement with the dilution method is not substantially better than that shown in Table III.

TABLE V

COMPARISON OF RESULTS OBTAINED WITH KENT-JONES AND DUBOSCQ COLORIMETERS, USING GASOLINE SOLUTIONS OF INCREASING DILUTION

(5.6 pH phosphate buffer solution with cobalt-chromate)

	Kent-Jones colorimeter			Duboscq colorimeter	
	I	II	III	IV	V
	cc. used	Gasoline value	Gasoline value original extract	Gasoline value	Gasoline value original extract
			Flour D		
Original extract	8.0	1.60	1.60	1.70	1.70
75 cc. orig. extr. 25 cc. gas.	6.5	1.30	1.72	1.35	1.80
50 cc. orig. extr. 50 cc. gas.	4.5	0.90	1.80	0.95	1.90
25 cc. orig. extr. 75 cc. gas.	2.2	0.44	1.76	0.55	2.20
10 cc. orig. extr. 90 cc. gas.	0.9	0.18	1.80	0.20	2.00
			Flour E		
Original extract	4.8	0.96	0.96	1.00	1.00
75 cc. orig. extr. 25 cc. gas.	3.6	0.72	0.96	0.82	1.09
50 cc. orig. extr. 50 cc. gas.	2.4	0.48	0.96	0.48	0.96
25 cc. orig. extr. 75 cc.	1.15	0.23	0.92	0.30	1.20
10 cc. orig. extr. 90 cc. gas.	0.45	0.09	0.90	0.15	1.50

Visser't Hooft and de Leeuw, in advancing their theory of H^+-ion concentration change said:

> "Kent-Jones' method uses the standard solution in different concentrations. In some cases up to 15 cc. of the standard solution is diluted with 40 cc. water and in other cases only 2 cc. of the standard solution is diluted with the same amount of water. Holger Jørgensen (1927) has shown that the pH of the chromate solutions has a big influence on the tint of these solutions. He therefore dilutes his inorganic standard solution with strongly buffered solutions, thereby keeping his pH constant and the tint of his solution the same."

It did not appear to the writers that this was a feasible explanation and they therefore estimated the H^+-ion concentration of water after the varying typical additions of standard solution they suggested in 1927. Table VI gives the results obtained, using both tap water and distilled water. The determinations of H^+-ion concentration were obtained using the quinhydrone electrode and the method described by Kent-Jones (1927, pp. 374-377). It will be seen from this table that there is no justification for the explanation suggested by Visser't Hooft and de Leeuw.

TABLE VI

H^+-Ion Concentration of Kent-Jones and Herd's Cobalt-Chromate Standard Solutions, when Diluted with Different Quantities of Water

	Tap water	Distilled water
	pH	pH
Water	7.15	5.68
1 cc. standard plus 50 cc. water	7.15	5.52
5 cc. standard plus 50 cc. water	7.15	5.60
10 cc. standard plus 50 cc. water	7.13	5.60
20 cc. standard plus 50 cc. water	6.98	5.64
50 cc. standard plus 50 cc. water	6.95	6.00
Standard solution	7.05	6.08

In endeavoring to obtain some light on this problem the authors used the Duboscq and the Kent-Jones colorimeters, observing the column of liquid at various depths. Table VII shows the results obtained on a number of flours using the aqueous cobalt-chromate solution as suggested by Kent-Jones and Herd (1927), the extracts being examined at various depths. Column I shows the results obtained [illegible] Duboscq colorimeter at a depth of 1 cm. and column II those when the reading was taken at a depth of 5 cm., the necessary ca[illegible]ction being made to reduce the results to a proper level [illegible]nparison. Column III similarly gives

the results obtained in the Kent-Jones colorimeter using 20 cc. and column IV using 50 cc. It will be seen from this table that both colorimeters show a discrepancy with varying depth; depth, or intensity of color, is the controlling factor and not the H^+-ion concentration, which is essentially the same in all instances. In the Duboscq method there can obviously be no change in this property, the same solution is merely being used at different depths.

TABLE VII

VARIATION OF GASOLINE VALUE WITH DEPTH OF EXTRACT USING BOTH KENT-JONES AND DUBOSCQ COLORIMETERS

(Aqueous cobalt-chromate solution)

Sample	Duboscq colorimeter		Kent-Jones colorimeter	
	I Gasoline value (1 cm. depth)	II Gasoline value (5 cm. depth)	III Gasoline value (20 cc.)	I Gasoline value (50 cc.)
101	1.30	1.53	2.25	2.40
102	1.00	1.20	1.50	1.80
103	1.00	1.10	1.50	1.80
104	0.65	0.86	1.10	1.30
105	0.65	0.88	1.25	1.40
106	0.60	0.86	1.15	1.40
107	0.50	0.62	0.90	1.10
108	0.40	0.48	0.60	0.80

Table VIII shows similar results obtained using the buffer standard color suggested by Visser't Hooft and de Leeuw. It will be seen from this table that the results are more satisfactory, altho, as previously shown in Table VII, this cannot be due to variation in H^+-ion concentration. On the whole, the values given by the Kent-Jones colorimeter are a little higher than those given by the Duboscq.

TABLE VIII

VARIATION OF GASOLINE VALUE WITH DEPTH OF EXTRACT, USING BOTH THE KENT-JONES AND THE DUBOSCQ COLORIMETERS

(5.6 pH phosphate buffer with cobalt-chromate)

Sample	Duboscq colorimeter		Kent-Jones colorimeter	
	I Gasoline value (1 cm. depth)	II Gasoline value (5 cm. depth)	III Gasoline value (20 cc.)	IV Gasoline value (50 cc.)
101	1.30	1.36	1.50	1.50
102	1.10	1.06	1.15	1.10
103	0.90	0.96	1.15	1.10
104	0.70	0.75	0.85	0.90
105	0.75	0.80	0.90	0.90
106	0.65	0.72	0.85	0.90
107	0.55	0.58	0.75	0.70
108	0.40	0.45	0.50	0.50

Work was continued on this problem using both colorimeters, and when potassium chromate alone was used there was considerable difficulty in matching colors. The flour extracts have a decided red tint. This red tint could be imparted to the standard solution in various ways, for instance, by the use of cobalt nitrate, as originally suggested by Kent-Jones and Herd (1927), or by the use of an acid solution which resulted in the partial formation of red bi-chromate ions. Work along these lines showed some very interesting results. If cobalt nitrate be excluded and the red tint purely obtained by an acid chromate solution, excellent results could be obtained in both colorimeters at any depth. Table IX shows results obtained using the potassium chromate made up with an acid buffer solution, the H^+-ion concentration of which is equivalent to pH = 4.4. (50 cc. M/5 potassium hydrogen phthalate + 7.50 cc. M/5 sodium hydroxide, diluted to 200 cc. according to Clark and Lubs). This standard gives just about the right color for comparison. It will be observed that no cobalt is present.

TABLE IX

VARIATIONS OF GASOLINE VALUE WITH DEPTH OF EXTRACT, USING BOTH THE KENT-JONES AND THE DUBOSCQ COLORIMETERS

(Buffer solution of pH=4.4 without cobalt)

Sample	Duboscq colorimeter		Kent-Jones colorimeter	
	I Gasoline value (1 cm. depth)	II Gasoline value (5 cm. depth)	III Gasoline value (20 cc.)	IV Gasoline value (50 cc.)
K/k 30	1.65	1.66	1.80	1.70
K/k 31	1.50	1.50	1.60	1.60
P/p 1	1.50	1.50	1.55	1.50
P/p 2	1.05	1.05	1.10	1.10

When the phosphate buffer solution (pH = 5.6) is added to the Kent-Jones and Herd original coloring solution containing cobalt, a precipitate of cobalt phosphate may be formed, as will be seen later. On the other hand, the acid solution will certainly contain some bi-chromate ions in addition to the cobalt ones. This may be one of the reasons for the usefulness of the phosphate buffer solution in this case. Further interesting results were obtained in comparing the same standards at different depths. For instance, the cobalt-chromate standard in water was used in the Duboscq colorimeter. Five cc. of this solution (that is, 10 cc. of 0.5 per cent chromate solution and 1.5 cc. of 10 per cent cobalt solution made up to 100 cc.) was diluted to 10 cc. and used in one limb of the Duboscq. The other limb contained the undiluted standard. The half-strength solution was set at 1, 2, and 5 cm. depths. For

matching, one would naturally expect that the full standard would be at exactly half the depth. In this case the readings were found to be correct in the first two instances, namely, 0.5 and 1.01 cm. In the case where the diluted standard was set at 5 cm., a figure of 2.30 cm. was obtained instead of the expected 2.50 cm. Similar experiments were carried out using the buffered cobalt-chromate solution as suggested by Visser't Hooft and de Leeuw and also using the solution with pH = 4.4, which contains no cobalt. Turbidity is not invariably caused by the addition of the pH = 5.6 phosphate buffer, as advised by Visser't Hooft and de Leeuw, to the aqueous cobalt-chromate solution. The formation of the precipitate seems to depend upon the speed with which the buffer solution is added. Turbidity is usually obtained by adding to the 10 cc. of 0.5 per cent potassium chromate with 1.5 cc. cobalt nitrate solution a few cc. of phosphate buffer and thoroughly mixing —the dilution to 100 cc. being made by further small additions. If the buffer solution be added in bulk quickly up to the 100-cc. mark, and the mixing then effected, the mixture remains practically clear. These two standards give different results in the two arms of the Duboscq apparatus using the half dilution method. These results are shown in Table X. It will be observed from this table that there is a considerable deviation from the expected result in the case of the buffered cobalt-chromate solution when turbid, but excellent results are obtained when clear and also with the pH = 4.4 buffer without cobalt.

TABLE X

COMPARISON OF DIFFERENT CONCENTRATIONS OF THE SAME TINTED SOLUTIONS, USING THE DUBOSCQ COLORIMETER

Depth of 0.5 strength solution	Depth of unit strength solution					
	I	II		III	IV	V
	Aqueous cobalt-chromate solution	Buffered chromate solution pH=5.6		Buffered solution no cobalt pH=4.4	Aqueous cobalt-chromate +bi-chromate	Buffered cobalt-chromate sol. pH=6.4
		Turbid	Clear			
cm.	cm.	cm.	cm.	cm.	cm.	cm.
1.0	0.50	0.45	0.50	0.48	0.50	0.50
2.0	1.01	0.90	1.00	0.99	1.00	1.00
5.0	2.30	2.05	2.45	2.45	2.42	2.25

This seems to indicate that with varying depth and intensity some absorption effect is manifesting itself. Apparently more than one factor cause unexpected results in this experiment. In the case of the turbid buffered cobalt-chromate solution, these deviations appear to be due to the presence of the fine precipitate.

With the aqueous cobalt-chromate solution (no buffer present) which is quite clear, the deviation may be due to variations in absorption of red and yellow light at various depths and intensities. This would point to the red cobalt ion being responsible for the errors observed. As will be seen from Table X, column III, no deviation occurs with the 4.4 pH buffer (no cobalt present). The red tint here is due to the formation of the bi-chromate ion. It might reasonably be expected that the presence of the red bi-chromate ion would not have the same effect as the red cobalt ion. There would probably not be so marked a difference in the absorption of two closely related ions such as chromate and bi-chromate as in the case of a chromate and a cobalt ion. It would be difficult to prove this point without an elaborate research into the absorption effects of various ions in mixtures, but on general grounds it is not surprising that the pH = 4.4 buffered solution gives more reliable results than the aqueous cobalt-chromate solution. There is no doubt, also, that when no red tint is present there is a tendency to use higher quantities of the yellow coloring matter. It will be seen that with both the Duboscq and the Kent-Jones colorimeters complete agreement is obtained with the pH = 4.4 buffered chromate solution and that differences of depth and concentration do not interfere. It is, therefore, suggested that the theory propounded by Visser't Hooft and de Leeuw—that the error is caused by a variation in the H^+-ion concentration of the standard solution due to varying amounts of the standard color being used—is wrong, and that the trouble is possibly mainly one of variation in red and yellow absorption. That is, a mixture of chromate and cobalt probably causes varying absorption depending on the depth of liquid under examination. If the tint of the solution being used for the comparison is not the same, errors occur in matching, the greater the depth, the greater the error. If the phosphate buffered cobalt-chromate standard becomes cloudy, an additional factor is introduced. If it remains clear, it seems probable that the presence of the red bi-chromate ion largely eliminates the error caused by the red cobalt ion. This is indicated in Table X, column IV, where it is seen that the addition of bi-chromate ions to an aqueous cobalt-chromate solution which gives incorrect results with the half dilution method, reduces the error appreciably. It will also be seen from column V that if a buffer solution of lower acidity (pH =6.4) is used, bi-chromate ions are apparently not produced in sufficient quantity and the

error persists. In order, therefore, to obtain strictly concordant results between the use of the Kent-Jones and the Duboscq colorimeters, it is best to use the chromate solution of pH = 4.4 without cobalt. Figure 1 shows how results obtained in the Kent-Jones colorimeter, using the original aqueous solution of cobalt-chromate suggested, may be corrected for the chromate solution of pH = 4.4, if this is desired.

At first sight it may seem surprising that the dilution experiments given in Tables III and V give the results they do. Dilution, in this instance, does not cause unexpected results. It should be remembered, however, that the ratios of red and yellow are fixed. This is not the same as the examination of different flour extracts, intensities of which probably vary in unknown rates of red and yellow.

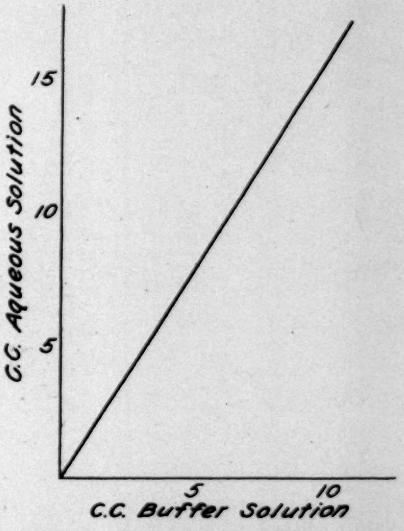

Fig. 1. Conversion of the Readings Using Aqueous Cobalt-Chromate Solution to Those Using 4.4 pH Buffer Solution

The practical result of applying this correction is to compress the scale. The numerical method suggested by Kent-Jones and Herd for recording the yellowness of flour was brought out as a practical guide for millers. The extended scale, using the original solutions, is therefore of distinct value. Altho the use of the Duboscq colorimeter gives a more accurate estimation of the content of carotin, from the point of view of distinguishing bleached and unbleached flours, the extended scale given by the aqueous solution of cobalt-chromate in the Kent-Jones colorimeter is pre-

ferred. For the purpose of comparison, Figure 1 enables the necessary correction to be easily applied. A typical figure, using the Kent-Jones and Herd scale, for unbleached flour is 13.5 and for a bleached flour about 6.0. This would correspond on the corrected scale to approximately 8.5 and 4.0. For the guidance of millers this is a restricted range.

Grade Determination

The most important part of the work of Kent-Jones and Herd (1927) was the alkaline methyl alcoholic extraction giving information as to the grade, or bran contamination, of the flour. Probably partly owing to the lower intensity of color, the agreement between the two colorimeters is better in this case. It may also be that the color extracted from the flour has a mixture of red

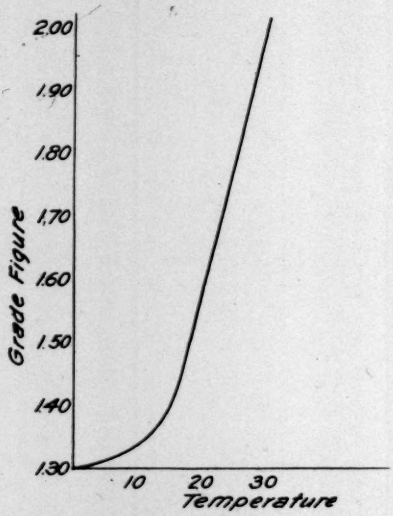

Fig. 2. Effect of Temperature on Methyl Alcohol Value (Patent Flour)

and yellow more in agreement with the standard tinting solution recommended, and hence on dilution, etc., errors due to variation in absorption are not so pronounced. Visser't Hooft and de Leeuw preferred the standard made up without cobalt but buffered at pH = 5.6. This presumably contains some bichromate ions, which may contribute a slight red tint to the solution. The authors find that this is insufficiently red for use in the Kent-Jones colorimeter, but can be used in the Duboscq, owing to the low intensity of color in the one centimeter depth of extract. The criticisms of

Visser't Hooft and de Leeuw on this, the most important branch of the work, were of less serious consequence. They suggested that it was impossible to effect a thoro extraction of the coloring matter from the bran in the time given (16 hours), and that in this period only part of the coloring matter was extracted; an analysis of the figures they give suggests this as approximately 60 per cent.

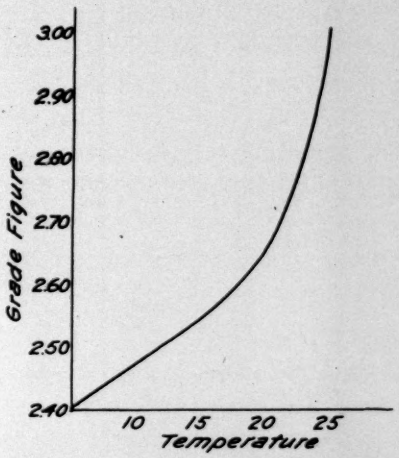

Fig. 3. Effect of Temperature on Methyl Alcohol Value (Lower Grade Flour)

The authors, therefore, repeated the work on a number of flours, the times of extraction being 3, 16, and 40 hours. The last period was taken, as the Dutch investigators suggest that this is the time necessary for complete extraction. When the 3-hour method was used the flour was allowed to stand with alkali about half an hour and then the methyl alcohol was added. The procedure was then carried out as usual. Table XI shows the results obtained, and altho there is a slight tendency for the results at 40 hours to be higher, it is not of great practical importance.

TABLE XI

EFFECT OF TIME OF EXTRACTION UPON THE METHYL ALCOHOL FIGURE

Results are shown as number of cubic centimeters of 0.005% chromate solution equivalent to 1 cm. depth of extract.

Flour	Time of extraction		
	3 hours	16 hours	40 hours
Straight run	2.00	2.10	2.40
Patent	1.40	1.40	1.60
A 211	1.60	1.60	1.60
A 212	2.40	2.60	2.60

Visser't Hooft and de Leeuw also emphasized the importance of temperature with this method of extraction. In flour work generally the temperature of extraction is an important matter, but as a rule the variation has to be over 10°C. for there to be a significant difference. Two flours were, therefore, extracted at 0°, 10°, 15°, 20°, and 30°C. The results are shown in Table XII and graphically in Figures 2 and 3. It will be noticed, however, that even the comparatively large range from 10° to 20°C. makes a difference of little more than one point on the Kent-Jones and Herd scale and is therefore of little significance commercially.

TABLE XII

EFFECT OF TEMPERATURE OF EXTRACTION UPON METHYL ALCOHOL VALUES, EXPRESSED AS NUMBER OF CUBIC CENTIMETERS OF 0.005% CHROMATE SOLUTION EQUIVALENT TO ONE CM. Depth of Extract

	Temperature of extraction				
Flour	0°C.	10°C.	15°C.	20°C.	30°C.
A 211 (patent)	1.30	1.34	1.40	1.60	2.00
A 212 (low grade)	2.40	2.47	2.54	2.65	3.00

Fairly wide variations were encountered by various operators in using the Duboscq colorimeter. Visser't Hooft and de Leeuw remarked that the colorimeter designed by Kent-Jones and Herd was less tiring to the eye than the Duboscq. The authors have found this so to a marked degree and there is no doubt that this form of colorimeter gives more consistent results with various observers. In general, the authors do not consider that the depth of one cm. is sufficient for an accurate comparison of color in the Duboscq with many of the gasoline and alkaline methyl alcohol extracts of flours. This is well brought out by Tables XIII and XIV. In Table XIII are given the results of five independent operators on the gasoline value of flours using both the Kent-Jones and the Duboscq colorimeters, the results again being expressed both on the numerical scale suggested by Kent-Jones and Herd and as the number of cubic centimeters of standard aqueous cobalt-chromate solution per one cc. of extract. It should be remembered that in this case the difficulty of comparison is aggravated; besides the fact that compound colors of red and yellow are being looked at, they are also in different solvents, i.e., water in one case and gasoline in the other.

TABLE XIII

READINGS OBTAINED BY DIFFERENT OBSERVERS ON THE SAME FLOUR EXTRACTS, GASOLINE VALUE (KENT-JONES AND DUBOSCQ COLORIMETERS)

Observer	cc. used in Kent-Jones method		Gasoline value Kent-Jones colorimeter		Gasoline value Duboscq colorimeter
	As taken	Corrected	As taken	Corrected	
		Flour P			
1	6.5	4.1	1.3	0.82	0.80
2	6.0	3.75	1.2	0.75	0.78
3	6.0	3.75	1.2	0.75	0.65
4	6.5	4.1	1.3	0.82	0.60
5	6.0	3.75	1.3	0.75	1.00
		Flour Q			
1	15.5	9.75	3.1	1.95	1.60
2	16.0	10.0	3.2	2.00	1.60
3	15.5	9.75	3.1	1.95	1.45
4	15.0	9.25	3.0	1.85	1.35
5	15.0	9.25	3.0	1.85	1.80

TABLE XIV

READINGS OBTAINED BY DIFFERENT OBSERVERS ON THE SAME FLOUR EXTRACTS, METHYL ALCOHOL VALUES (KENT-JONES AND DUBOSCQ COLORIMETERS)

Observer	cc. used in Kent-Jones method	Methyl alcohol values Kent-Jones colorimeter	Methyl alcohol values Duboscq colorimeter
	Flour P		
1	9.5	1.95	2.70
2	9.5	1.95	2.80
3	9.5	1.95	2.00
4	9.5	1.95	2.40
5	9.5	1.95	2.20
	Flour Q		
1	6.5	1.30	1.70
2	7.0	1.40	1.80
3	6.5	1.30	1.30
4	6.5	1.30	1.20
5	7.0	1.40	1.60

Conclusions

In view of the criticisms of Visser't Hooft and de Leeuw on the numerical method for the estimation of the color of flour suggested by Kent-Jones and Herd (1927), a critical survey was made of the method. The initial observation of Visser't Hooft and de Leeuw that the Kent-Jones and Herd method gives results not in agreement with the Duboscq colorimeter, was confirmed. The suggestion of Visser't Hooft and de Leeuw that this was due to a change of H^+-ion concentration of the solution used for comparison owing to the varying amounts of standard solution used, was found to be wrong, and it is suggested that the discrepancy may be due to certain absorption effects of red and yellow light.

To obviate the possible varying absorption caused by the cobalt-chromate mixture, it is suggested that an acid chromate solution be used, as apparently the absorption effects of chromate and bichromate ions are more nearly similar. For practical purposes, however, it is suggested that the numerical standard given by Kent-Jones and Herd (1927) is more useful. Little confirmation could be found of the criticism advanced by Visser't Hooft and de Leeuw concerning the extraction method employed in obtaining the alkaline methyl alcohol extracts. Confirmation was obtained of the Visser't Hooft and de Leeuw statement that the colorimeter designed by Kent-Jones and Herd is less tiring to the eye than the Duboscq and this results in a closer agreement being obtained by various observers with this colorimeter than with the Duboscq.

The authors' thanks are due to A. J. Amos, who not only helped in making many of the determinations reported, but who assisted generally with many valuable suggestions.

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EFFECT OF DRY SKIMMILK ON THE FERMENTATION AND HYDROGEN-ION CONCENTRATION OF DOUGHS[1]

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It appears to be generally conceded that the inclusion of milk solids in bread tends to enhance its nutritive value. As butter is commonly spread on bread served on American tables, the non-fat solids of milk, or, in other words, the solids of skimmilk, advantageously supplement the other nutrients of ordinary milk-free bread. Natural, or whole, milk and fluid skimmilk have been extensively used as part or all of the liquid portion of bread doughs in many bakeries. During the last score of years notable advances have been made in the development of processes for the manufacture of dry milk, and of dry skimmilk. Certain obvious advantages are attached to the use of these dried products in comparison with the original material, provided they possess the normal properties of the fresh material on reconstituting them with water. It was with a view toward determining certain effects resulting from superimposing dry skimmilk upon the ordinary milk-free formula that these studies were undertaken. The properties studied have been concerned particularly with colloidal behavior of the dough, progress of fermentation, and the bread "score" based upon size, texture, flavor, and color, rather than with nutritive values.

The views entertained prior to 1925 relative to the properties of flour and of dough that are reflected in the characteristics of the resulting bread have been reviewed by Bailey (1925) and need not be repeated. Hydrogen-ion concentration, in its relation to colloidal behavior of and enzyme activity in dough has been discussed recently from several viewpoints. Bailey and Johnson (1924) took issue with the assumption that all doughs should be fermented to the same H-ion concentration, and suggested that the sudden acceleration of the discharge of CO_2 from dough marks a critical state in the control of the fermentation. They described a device for measuring the rate of gas production in and loss of gas from dough.

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Emphasis should be laid upon the significance of CO_2 production in or "gassing" of doughs. Wood (1907), Humphries and Simpson (1909), and others since that time have called attention to the necessity of a continued high level of CO_2 production in dough, to insure the production of yeast-leavened bread of good quality. The production of CO_2 is conditioned by the original composition of the dough with its fermentable sugars, yeast nutrients, and enzyme accelerants (including H-ions), and also by the products of enzyme activity in the dough during fermentation. Prominent among the enzymes that function in the latter connection is diastase.

Phosphates appear to be either essential to, or active in alcoholic fermentation. This has been stressed by Harden and Young (1906), who proposed equations which involved phosphate as an essential component of the reactions of alcoholic fermentation. Whether or not this equation is valid, the fact appears to be abundantly demonstrated that the addition of phosphates to a fermentation medium deficient in that constituent will result in an acceleration of the fermentation process.

Phosphates have long been known to be a characteristic constituent of milk. Lenstrup (1926), in the analysis of fifteen samples of normal cows' milk, found 95.4 mgm. of total phosphorus per hundred cubic centimeters of milk. Of this 17.1 mgm. was acid insoluble phosphorus that was almost entirely casein phosphorus with a trace of lipoid phosphorus; 78.3 mgm. was acid soluble phosphorus, of which 67.1 mgm. was inorganic and 11.2 mgm. organic phosphorus.

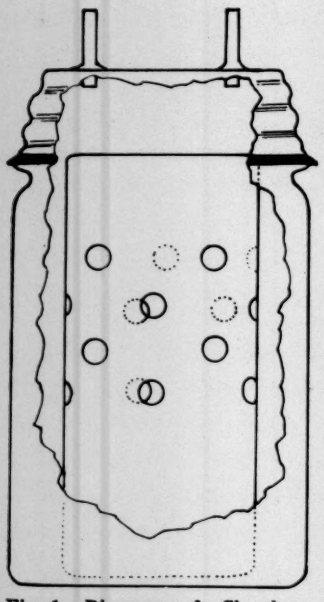

Fig. 1. Diagram of Chamber and Beaker in Which Doughs Were Fermented

Experimental

The first stage of this study involved measurements of the relative rate of fermentation in doughs prepared with and without dry skimmilk and fluid skimmilk. The method described by Bailey and Johnson (1924) for measuring the rate of formation of gaseous CO_2 in yeast-leavened doughs and loss of CO_2 from them was followed, except for one modification of the dough container. An aliquot of the freshly mixed dough to be studied was placed in a special glass beaker 5x11.5 cm.,

which was just large enough in diameter to fit comfortably into a pint mason jar and tall enough to be flush with the top of the jar. This beaker was made from glass tubing having an internal diameter of 5 cm. The sides of the upper half were perforated with twenty-eight holes about 0.5 cm. in diameter. This specially blown beaker was found more convenient than the shallow beaker and perforated waxed paper cylinder originally used with this method. A diagram of the entire device appears in Figure 1.

The baking formula was as follows:

	Grams
Flour	350.00
Cane sugar	10.50
Salt	6.13
Yeast	10.50
Shortening	5.25
Water	sufficient

These ingredients were mixed for 4 minutes in a mechanical dough mixer.

An aliquot of the dough equivalent to 40 grams of flour was used.

The flour used in these experiments was a standard patent milled from hard spring wheat by the Minnesota State Experimental Mill, in Minneapolis. The flour (No. 2) contained 13.6% moisture, 0.5% ash, and 12.7% protein. The average loaf volume, as determined in the regular or control baking tests, was 2200 cc. and the color and texture scores were high.

The dry skimmilk used was from a sample furnished by the American Dry Milk Institute; the fluid skimmilk was obtained from the University creamery from day to day as needed.

One series included doughs containing fluid skimmilk equivalent to 1/3 of the amount of water which would be used in a non-milk dough, together with sufficient water to produce a dough of normal consistency. In other doughs in this series no water was used, but all the liquid added was skimmilk. The former is referred to as a 1/3 skimmilk dough and the latter as a full skimmilk dough.

The second series included sufficient dry skimmilk to give skimmilk solids equivalent to the previous sets, thus making a 1/3 and a full skimmilk dough, or in terms of percentage of dry skimmilk, they contained about 2% and 6%, respectively, of this ingredient superimposed upon the regular formula. The dry skimmilk was "reconstituted" before adding it to the dough by allowing it to stand for one hour, with frequent shaking, with distilled water.

Figure 2 records graphically the results of observations of control or milk-free doughs and doughs in which fluid skimmilk replaced the

water used in the control doughs. Results obtained with 1/3 and 2/3 fluid skimmilk were practically identical with those recorded in this figure. It appears from the graphs that fluid skimmilk had comparatively little effect upon the fermentation. For about 170 minutes the total volume of gas evolved (curves A) was about the same for both doughs. After this the volume of gas produced by the milk dough was slightly in excess of that produced by the control. Since the dough expansion (curves B) was very nearly the same for the control and the milk doughs, it follows that the loss of carbon dioxide from the two was practically the same up to 170 minutes, after which the loss from the doughs containing milk was slightly greater (curves C). After 220 minutes the volume of carbon dioxide lost exceeded the volume expansion of the dough.

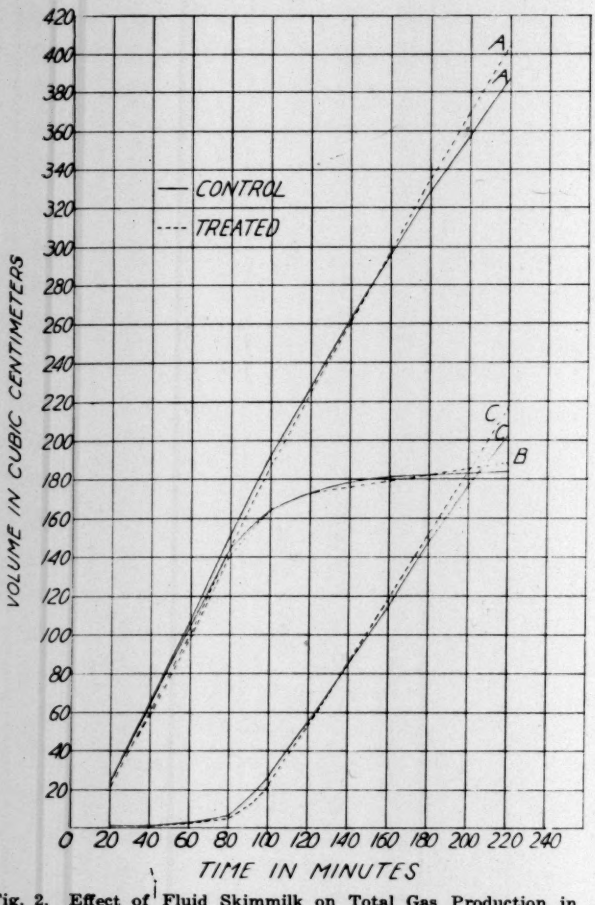

Fig. 2. Effect of Fluid Skimmilk on Total Gas Production in the Fermenting Dough (Curves A), Volume of Dough (Curves B), and Loss of CO_2 from Dough (Curves 3)

In the observations on doughs prepared with dry skimmilk recorded in Figures 3 and 4, it appears again that the expansion was practically the same for both the milk and the control doughs. We find, however, that the total volume of gas produced was greatest in the doughs containing milk. As the gas retention (curves B) for all the

doughs is practically the same, it follows that the larger amount of additional gas produced by the doughs prepared with milk is lost from the dough.

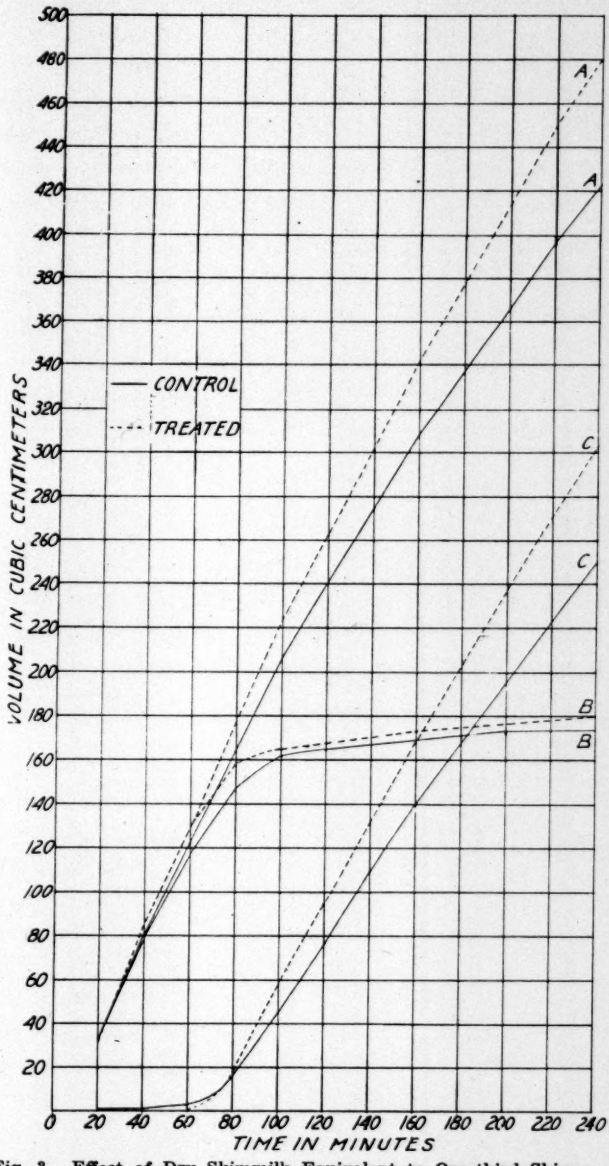

Fig. 3. Effect of Dry Skimmilk Equivalent to One-third Skimmilk on Total Gas Production in the Fermenting Dough (Curves A), Volume of Dough (Curves B), and Loss of CO_2 from Dough (Curves C)

With this series of doughs the total amount of carbon dioxide lost from the milk-free dough averaged about 210 cc., or 0.412 grams, in the 240-minute period. This weight of carbon dioxide plus an equivalent weight of alcohol would give a total loss of 0.84 gram from 40 grams of flour, or a fermentation loss of 2.1%. This agrees very closely with Snyder's results (1903). The average loss from the dry skimmilk dough was 288 cc., or 0.566 grams, and with the equivalent of alcohol the total loss was 2.9%.

The reasons for the effect of the dry skimmilk in increasing the rate of fermentation are not clear from the data presented here. Several possible hypotheses may be suggested. Dry skimmilk, on ignition,

yields about 8% of ash rich in phosphorus compounds and its addition to dough would increase the amount of mineral matter and of phosphorus in the dough. In view of the work of Swanson, Willard, and Fitz (1915), and Harden (1923), it is possible that the increased rate of fermentation noted in the doughs to which dry skimmilk was added was influenced, in part at least, by the addition of phosphates carried by the milk. The effect in this case did not seem to be on the length of the fermentation period, as was noted by Swanson, Willard, and Fitz, since the curves presented do not indicate a substantial difference in the length of fermentation period between the control and the milk doughs.

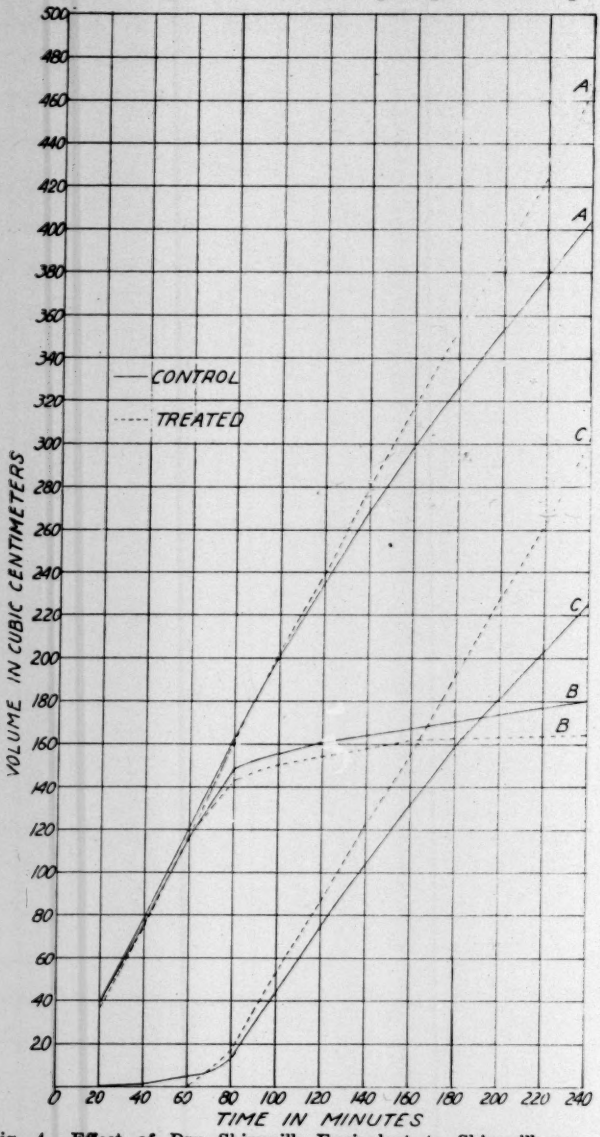

Fig. 4. Effect of Dry Skimmilk Equivalent to Skimmilk as the Entire Liquid in Fermenting Dough on the Total Gas Production (Curves A), Volume of Dough (Curves B), and Loss of CO_2 from Dough (Curves C)

While our results may be in accord with Harden's observations to the extent that there is an increase in the rate of fermentation, they do not accord with the quantitative relation between the phosphates added in the milk and the amount of carbon dioxide produced which

appeared in his work. Thus, the increase in carbon dioxide production was approximately the same whether 2, 4, or 6% of dry skimmilk was added. This suggests that the effect of the dry skimmilk in increasing the gas production is influenced by factors other than the quantity of phosphate added in the dry skimmilk.

Dry Skimmilk and the H-ion Concentration of Dough

In the discussion of the influence of H-ion concentration on the properties of dough and of bread, the opinion has been expressed that the increase in degree of acidity normally occurring in fermentation is advantageous in the aggregate. Grewe and Bailey (1927) pointed out that there is no evidence of an improvement of the physical properties of dough with this increase in acidity. They observed, as did Rumsey (1922), that the activity of flour diastase increased with the increase in acidity, and it is possible that the activity of the yeast enzymes may be coincidentally accelerated. (See Euler and Emberg, 1919.) Such advantages as accrue from the increased acidity in fermentation of doughs are probably the consequence of accelerated fermentation rate, rather than the modification of gluten properties. In a dough in which the enzyme activities are maintained at or above the normal level, superior bread may result from the baking of doughs which are less acid than the normal. This was demonstrated by Grewe and Bailey (1927).

TABLE I

HYDROGEN-ION CONCENTRATION OF FERMENTING DOUGHS CONTAINING VARYING PROPORTIONS OF DRY SKIMMILK

Dry skimmilk per 100 grams of flour	H-ion concentration of dough, as pH					Bread pH
	When mixed	After 1 hour	After 2 hours	After 3 hours	After 4 hours	
grams	Flour No. 3*, Dry Skimmilk No. 1					
0	5.55	5.47	5.41	5.32	5.28	5.56
2	5.77	5.66	5.60	5.54	5.47	5.67
4	5.88	5.80	5.76	5.68	5.60	....
6	6.01	5.87	5.83	5.77	5.77	5.83
	Flour No. 3*, Dry Skimmilk No. 2					
0	5.53	5.38	5.28	5.19	5.16	5.49
2	5.70	5.53	5.50	5.39	5.34	....
4	5.78	5.70	5.61	5.60	5.51	....
6	5.87	5.75	5.71	5.68	5.65	5.83
	Flour No. 1†, Dry Skimmilk No. 1					
0	5.54	5.38	5.28	5.25	5.18	5.43
2	5.71	5.55	5.49	5.43	5.36	....
4	5.82	5.72	5.68	5.60	5.56	....
6	5.89	5.77	5.78	5.70	5.68	5.84

Compositon of	Mosture, %	Crude protein, % (N x 5.7)	Ash, %
*Flour No. 3	13.80	11.5	0.43
†Flour No. 1	12.95	12.1	0.49

A series of doughs containing from 0 to 6 grams of dry skimmilk per 100 grams of flour were mixed and fermented at 28° C. The H-ion concentration of these doughs was determined at hourly intervals. Two lots of flour were used, one with a spray-process dry skimmilk, designated as No. 1, and the other with two brands of spray-process dry skimmilk, Nos. 1 and 2. The resulting data are recorded in Table I in terms of pH.

It is evident that the dry skimmilk buffers the dough appreciably and in approximate proportion to the quantity present. No difference between the two brands could be detected. The buffering effect registers on the freshly mixed dough, and in two of the three series, in terms of a reduced rate of change in pH during fermentation. The baked bread containing dry skimmilk was also lower in degree of acidity than the controls.

Rice and Markley (1924) demonstrated the buffer action of milk and attributed this property to the phosphates and the casein. Further evidence of this buffering action resulted from a simple experiment. When 30 grams of patent flour was suspended in 70 cc. of water the H-ion concentration was equivalent to pH = 5.95. Then the same weight of flour plus 6% of its weight (1.8 grams) of dry skimmilk was suspended in an equivalent quantity of water, and the H-ion concentration was then equal to pH = 6.30.

In spite of the fact that the doughs containing dry skimmilk are less acid than the controls at the end of 4 hours, the fermentation rate is at a higher level. This appears to be contrary to our assumption that increasing acidity, other things being equal, would tend to accelerate fermentation. There must be some compensating factor, or property of the milk, that offsets the effect of the lower acidity in maintaining a higher fermentation rate. With fermentation at or above the normal, or control, it is conceivable that the buffer effect is advantageous rather than otherwise, as the physical properties of the dough are modified or disturbed less in the presence of the buffers. This, in fact, may explain the greater "stability" that has been ascribed to doughs containing dry skimmilk, since stability implies that the system is in a less critical state, so far as the changes with the lapse of time are concerned.

Summary

Production of total gaseous carbon dioxide in yeast-leavened doughs was increased when dry skimmilk was superimposed upon the control formula. In this particular, its effect was greater than that of the fluid skimmilk with which it was compared.

Loss of gaseous carbon dioxide from such doughs was increased somewhat when dry skimmilk was included in the formula.

Rate of increase in the volume or displacement of yeast-leavened doughs was practically the same, whether or not dry skimmilk was included in the formula.

Buffer action of dry skimmilk was appreciable, as shown both by the initial H-ion concentration of the freshly mixed doughs and by the relative rate of change (in pH) of control and milk-containing doughs. In view of the observed accelerated fermentation rate in the presence of dry skimmilk, it is possible that the greater stability of doughs containing this material may be the consequence of the slower change in H-ion concentration.

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A METHOD OF MEASURING COLOR IN BREAD[1]

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(Read at the Convention June, 1928)

In describing any object, definite terms are desirable. This is especially true in science. Lord Kelvin is said to have made the statement: "When you can measure what you are speaking about and express it in numbers, you know something about it; when you can not measure it, when you can not express it in numbers, your knowledge is of a meager and unsatisfactory kind." In bread, the color of crust and crumb is of interest to the cereal chemist. A reddish brown crust and a light creamy crumb are desired by the American consumer. A deep brown crust is associated with high sugar content and this, in turn, with diastatic activity, which may be correlated with flour strength and good loaf volume. Since the color of bread is so important, it is desirable to know how to measure color in order to describe it definitely.

There are two general methods of measuring color, one is a psychological method by which color is measured directly as it is seen by the eye; the other is a physical method by which the light waves that cause the color may be measured.

The generally used color terms are vague and incongruous. Baby-blue, apple-green, heliotrope, burgundy, and the like have for years done duty. The terms used for a single hue, such as beige, sepia, hazel, leafmold, soapstone, monkey skin, mother goose, sphinx, marrow glace, and russet certainly do not convey distinct meanings. A system is needed that not only gives a method of measurement but also a notation by which colors may be designated with exactness.

[1] This report is made possible by the co-operation of the Bureau of Agricultural Economics, U. S. Dept. of Agr., where the method of applying the Munsell system, including formulas and apparatus, has been developed by Miss Dorothy Nickerson, color technologist, in connection with the research program of the Division of Cotton Marketing and the standardization work of the Hay, Feed, and Seed Division.

The Munsell Color System[2]

A. H. Munsell, a portrait painter and teacher, while teaching at the Massachusetts Normal School in Boston, developed the Munsell color system so that he might more definitely teach the principles of color to his classes in color composition. The system is described by Cleland (1921) and Nickerson (1928).

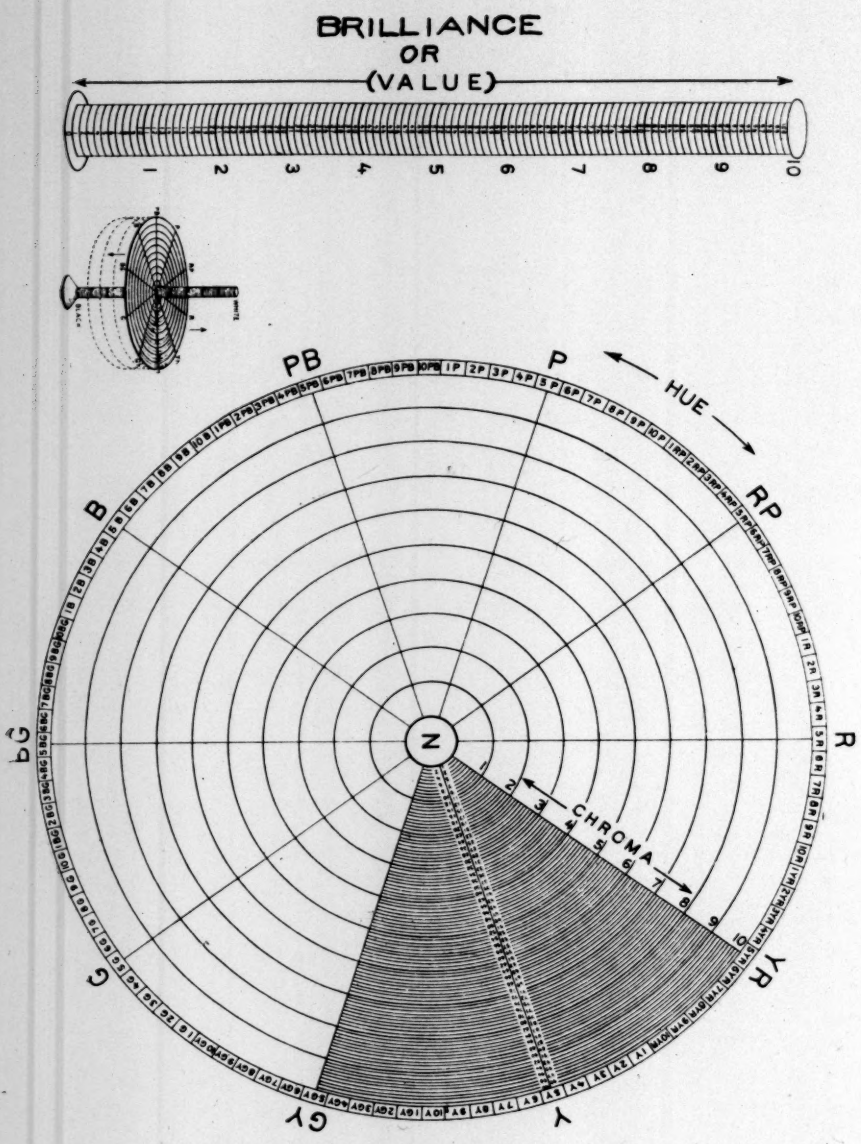
The system is based upon the psychological fact that color has three attributes: hue, brilliance, and chroma. Troland (1922), chairman of the colorimetry committee of the Optical Society of America, defines these three terms as follows: "Hue is that attribute of certain colors in respect of which they differ characteristically from a gray of the same brilliance and which permits them to be classed as reddish, yellowish, greenish, or bluish." "Brilliance (or value) is that attribute of any color in respect of which it may be classed as equivalent to some member of a series of grays ranging between black and white." "Saturation (or chroma) is that attribute of all colors possessing a hue, which determines their degree of difference from a gray of the same brilliance."

To illustrate the relation of the three color attributes a sphere or cylinder may be constructed, as illustrated in Figure 1. All color steps in any one direction are equal, to the eye, no matter in what part of the color solid they appear.

Hue.—The quality by which one color is distinguished from another, as a red from a yellow or a yellow from a green, is called hue. The five principal hues are red, yellow, green, blue, and purple; and the five intermediate hues are yellow-red, green-yellow, blue-green, purple-blue, and red-purple. These ten are known as the standard hues. In Figure 1 the initial letters of the colors are used to designate them (as R for red, YR for yellow-red, etc.). The standard hues are subdivided into 100 hues, and these may be still further subdivided if necessary. The notation follows the decimal system.

Hue may be represented by a letter or a number, or by both. Red, for instance, is R, but it may also be 5R. If only a number is used for hue it may be designated as follows: YR=15, Y=25, GY=35, etc. The letters help to designate the hue and separate the

[2] The Munsell system is being used in laboratory investigations on soils, cotton, hay, soap, fruits, vegetables, and meats. It is also used in the industries in testing paint, paper, ink, printing, and textiles.


BRILLIANCE
OR
(VALUE)
1
2
3
4
5
6
7
8
9
10
PB
P
RP
R
YR
Y
GY
G
BG
B
HUE
CHROMA
N

hue notation from the brilliance and chroma, though they may be omitted in any formula.

Brilliance.—The vertical axis of the cylinder consists of gradations from white to black through a series of grays, each step differing from the next by equal amounts. Absolute black, which is unattainable, is given a notation of 0/ and placed at the end of the scale; absolute white is given a notation of 10/ and placed at the top of the scale. All the grays fall between black and white and range from 1/ to 9/; 1/ is what is ordinarily called black, and is about the color of black velvet, while 9/ is the white ordinarily seen. The brilliance notation may be further subdivided by using decimals, giving 100 or even 1000 variations if it should be possible to see that many. Brilliance or value is illustrated by the upright diagram in Figure 1.

Chroma.—The hues red, yellow, green, blue, and purple are placed about the equatorial circumference of the color solid. If the chroma scale for any color is filled in toward the central gray core at the same brilliance at which it was started, it becomes less and less intense until it loses all color and becomes gray. If the chroma is extended outward from the gray core of the solid, the color becomes stronger until it reaches a maximum. Munsell (1905) likens this change in chroma to that of a leaf which, as autumn comes, gradually loses its chroma and fades to neutral gray. Not all hues have the same maximum chroma of strength. The strongest blue-green pigments have a chroma strength of 5, the brightest green is 7, the strongest yellow pigment is 9, while the red and purple-blue extend out to 10. Compare a strong blue-green with a strong red and it is readily seen that the red is twice as strong as the blue-green. In order to divide the notation for value from the notation for chroma, a line or bar is used as in a fraction $\frac{8}{9}$ of $8/9$. Value invariably precedes chroma.

The idea of psychologically equal and comparable difference furnishes the basis for an ideal system for classifying and accurately recording any color. All systems based on it will be uniform, and colors may be specified without any reference to samples that may fade, or to formulas in which the value of the constituents may alter.

A color identification may be made to any accuracy required. For instance, when the color of a deep brown crust is compared with the crumb of the same loaf, the relationship for brilliance will probably be 6.20/ to 8.20/. The crust is redder in hue than

the crumb, perhaps in the ration of 18 to 25; and the crust color is considerably stronger in chroma, possibly /5.20 to /3.50. Or another crumb color may be compared with the one already measured which is 25Y 8.20/3.50. If it has somewhat weaker color it may be represented by the value 25Y 8.32/3.22.

Apparatus

The Munsell apparatus is provided with discs made to represent the principal and intermediate hues, as red, red-yellow, yellow, etc., at each step in brilliance and chroma for the hues that can be produced in permanent pigments. Each disc is marked as to hue, brilliance, and chroma. The index also provides discs ranging from black to white. These are designated with the symbols 1/ to 9/ inclusive.

The color of an object is measured by spinning two or more hue discs and neutral discs on a motor shaft at a speed high enough to resolve the colors of the discs into a single color. A straight-line opening is made on the discs from the circumference to the center (Maxwell discs). When the discs are placed on the shaft for spinning, they are overlapped by means of these openings so that part of each disc is exposed at the surface. The proportions of the different colors of the visible sections can be adjusted until there is a perfect match between the object under test and the rotating discs.

Constant lighting conditions are essential to all careful color work. The objects under investigation should be placed on a container or rack at definite distances from the observer. The apparatus should be set up preferably in a north window or under a north skylight at an angle comfortable to view. The observer should stand or sit far enough from the discs that a direct line from disc to eye will be about four or five feet. In making a comparison of the bread and disc colors it is essential that an equal area of each be exposed to the eye. Comparisons of unequal color areas may often be misleading.

Recording Data

A calibrated disc graduated on its perimeter in 100 divisions may be used for measuring the amount of space occupied by each disc. After the colors are matched the disc is superimposed upon the color discs and readings of the exposed areas are taken. The sum of the hue disc and the neutral discs should equal 100.

The following formulas have been developed for general use in calculating hue, brilliance, and chroma:

$$\text{Formulas: Hue: } z - \frac{\Sigma (A_1 \times P_1)}{\Sigma (A_1 \times P_1) + \Sigma (A_2 + P_2)} (z - x)$$

$$\text{Brilliance: } \sqrt{\frac{\Sigma (A \times B^2_{1 \cdot 2 \cdot 3 \cdots})}{100}}$$

$$\text{Chroma: } \frac{\Sigma (A \times C_{1 \cdot 2 \cdot 3 \cdots})}{100}$$

Symbols A=Area
H=Hue
C=Chroma
B=Brilliance
P=B X C—Power number
x=first hue (clockwise)
x=second hue (clockwise)

Notation—HB/C.

The use of these formulas is illustrated in the experimental section.

Experimental

Ten samples of flour were used in this investigation. Samples 3, 6, and 9 were soft wheat flours, the others were hard wheat flours. The bread was made according to the formula method of procedure described by Blish (1928), which is now under investigation by the committee on baking test of the American Association of Cereal Chemists.

Crust color.—The crust color was first scored according to the judgment of the operator by giving the darkest a score of 1, the next darkest a score of 2, and so on with the ten loaves of bread. The crusts were then tested by means of the apparatus previously described. The results obtained by these procedures are reported in Table I. There is a close agreement on the hue and color as judged by the observer and those obtained by means of the apparatus.

The data on crust color from Sample 1, used to illustrate the use of the formulas under the heading "Recording data," are:

Notation	Per cent area
15 YR 6/8	42
25 Y 7/8	24
N 9.4/	3.5
N 5/	30.5

TABLE I

COMPARISON OF 10 FLOUR SAMPLES AS TO COLOR OF CRUST AND DIASTATIC ACTIVITY

Lab. No. of flour sample	Crust color	Diastatic activity as maltose per 10 gm. of flour	Munsell notation			Brilliance × chroma
			Hue	Brilliance	Chroma	
	score.	mgm.	scale of 100*	scale of 10†	scale of 10‡	36
9	10	79	21.02	7.31	4.96	36.26
3	9	78	20.50	6.95	5.64	34.75
6	8	58	20.98	8.18	5.64	46.13
1	7	80	19.00	6.13	5.28	32.36
7	6	104	18.53	6.01	5.52	33.17
4	5	188	18.12	5.96	5.44	32.42
5	4	135	17.96	5.68	5.28	29.99
2	3	106	18.18	5.58	4.48	25.00
8	2	137	17.46	5.30	4.40	23.32
10	1	186	16.61	4.91	3.66	17.97

The score on crust color was made by giving the darkest a score of 1, the next darkest 2, and so on to the lightest, which received a score of 10.
*15=Yellow-red. 25=Yellow.
†0=Absolute black. 10=Absolute white.
‡0=Neutral. 10=Maximum.

When Sample 1 was used, the data obtained were as follows:

$$\text{Hue} = 25 - \frac{42 \times (6 \times 8)}{42 \times (6 \times 8) + 24\ (7 \times 8)} - (25 - 15) = 19.6\ \text{YR}$$

$$\text{Brilliance} = \frac{(42 \times 6^2) + (24 \times 7^2)\ (.5 \times 9.42) + (30.5 \times 5^2)}{100} = 6.13$$

$$\text{Chroma} = \frac{(42 \times 8) + (24 \times 8)}{100} = 3.36$$

Brilliance × chroma is the strength or so-called Power number of a color. For example, a dark, strong color is not nearly so distinct to the eye as a light color of equal strength. The light, strong color can be seen at a greater distance and will stand out more clearly. A dark, strong red, R4/10, has a power number of 40, while a light, strong yellow, Y 8/9, has a power number of 72. It is a combination of brilliance and chroma which makes a color stand out to the eye. Value × chroma is also recorded in Table I.

Part of the color of the crust is due to caramelization of the sugar that is produced by diastase. Rumsey (1922) suggested a method for the estimation of diastatic activity that was used in the examination of these flour samples. By applying the Rumsey method, 10 grams of flour in water was digested for 1 hour at 27°C. Diastatic activity was inhibited at the end of the hour by acidulation, and the digestion mixture was defecated and clarified with sodium tungstate solution. Reducing sugars in an aliquot were determined by the Quisumbing-Thomas method and computed as equivalents maltose in milligrams per

10 grams of flour. The results obtained by this procedure are also shown in Table I. In general, the flours having the highest diastatic activity have the lowest hue and chroma.

Crumb Color.—Crumb color determinations were made according to the observations of the operator and also by the apparatus under investigation. These determinations are recorded in Table II. There was a very close agreement on the chroma as judged by observations and by means of the apparatus. Two readings were taken, one with the lower part of the loaf lying next to the rotating discs, the other with the top of the loaf lying next to the rotating color discs. The difference between the two readings is the result of reflection caused by grain. This suggests the possibility of using this or some similar means of obtaining a quantitative measurement of grain.

TABLE II

COMPARISON OF 10 FLOUR SAMPLES AS TO COLOR OF CRUMB

Lab. No. of flour sample	Crumb color	Munsell notation		
		Hue	Brilliance	Chroma
	score*	scale of 100†	scale of 10‡	scale of 10§
9	W	15	8.12	3.15
		15	7.95	3.24
3	Cr+	15	8.03	3.69
		15	7.93	3.92
6	Cr+	15	8.03	3.96
		15	8.02	3.96
1	Cr–	15	7.77	3.33
		15	7.79	3.56
7	Y	15	7.87	4.14
		15	7.81	4.23
4	Y+	15	7.97	4.55
		15	7.87	4.72
5	Y–	15	8.07	3.96
		15	7.99	4.14
2	Cr	15	8.19	3.60
		15	7.99	3.87
8	Cr–	15	7.92	3.15
		15	7.75	3.33
10	Cr	15	8.19	3.60
		15	8.04	3.69

*W=White. Cr=Creamy. Y=Yellow.
†15=Yellow-Red. 25=Yellow.
‡0=Absolute black. 10=Absolute white.
§0=Neutral. 10=Maximum.

The three attributes, hue, brilliance, and chroma, are not all always of importance in judging the color of any object. Hue and brilliance are the attributes having significance in judging color of crust, while chroma is of importance in judging crumb. In judging white grades of American cotton, hue and brilliance are significant, that is, the creamy, light-colored cottons are the better grades, while the gray, darker colored cottons are of lower grades. In judging hay, the element of hue is the most important, that is, the highest grade is the greenest hay regardless

of whether the hay is light or dark. In tomatoes, the three elements seem to change simultaneously, some tomatoes being a dark strong red with a somewhat red-purple hue, while others are lighter and weaker in color and quite yellow in hue.

The method and apparatus that are being developed in the Bureau of Agricultural Economics of the United States Department of Agriculture were used in making this study. Because of the necessity of using the apparatus in another laboratory, only a few tests were made. From these few observations it appears that the apparatus gives a good quantitative measurement of both crust and crumb color. The time consumed in making the observations is not beyond that which is in keeping with the needs of commercial laboratories.

Summary

A psychological method devised by A. H. Munsell, by which color is measured directly by the eye, is applied to testing the color of bread. The three attributes, hue, brilliance, and chroma, are the basis of this system.

The color of an object is measured by spinning two or more discs on a motor shaft at a high enough speed to resolve the colors of the discs into a single color. When the discs are placed on the shaft they are overlapped by means of a slit so that part of each disc is exposed at the surface. The proportion of the different colors of the visible portion may be adjusted until there is a perfect match between the object under test and the rotating disc.

Color of crust and crumb of bread may be measured by means of this system.

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A PRACTICAL METHOD OF PHOTOGRAPHING BREAD

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For several years, cereal chemists have tried by various means to reproduce visual records of loaves of bread, with a result more or less satisfactory. Some have tried marking with a pencil around the outside of a thin slice of bread. This shows the volume and symmetry of the loaf very well, but nothing of the texture, break, or shred. Others have tried to show the texture, using printer's ink in addition to marking the outline by putting on a thin coat of ink with brush and then stamping the loaf on paper. More or less trouble has been experienced in getting good reproductions, because of the interior shrinking and leaving the crust protruding in such a way that it is difficult to get a good impression of texture. The whole procedure is unsatisfactory.

A plate camera has been used with much better results. This produces a satisfactory visual record, except that it is impossible to reproduce the crumb color. However, the use of a plate camera has many disadvantages. When the source of light is sunlight it is impossible to get desirable results on dark days. Another objection is the time factor. By using a 6½ by 8½ plate it is possible to blank half of it and take two pictures on the same plate. With this arrangement much time is consumed to say nothing of the cost of developing and printing.

A photographic method was sought that would largely eliminate these objections. An apparatus designed by R. S. Herman has been in daily use here in the laboratory. It has practically overcome the cost and time factors, and produces a visual record that is entirely satisfactory. The apparatus consists of a Sept camera mounted in a fixed position, two 9-inch aluminum reflectors,[1] which focus the light from two 400-watt lamps upon the loaf, giving the same intensity of light each day regardless of natural light. (Fig. 1)

The machine uses 35 millimeter negative films with 250 exposures to the roll (17 feet of film) operating on a strong spring, and takes individual pictures. On the back of the camera an indicator shows how much film is yet unexposed. The cost of the roll ready to put into the camera is $1.00. The developing and printing cost is $4.20, making a total of $5.20, or about 2 cents per print. Having one-day

[1]Briggs Photo Supply Co.

service on developing and printing, there is little delay in completing the record. The time factor here is practically eliminated, as the pictures are taken as fast as one loaf is removed and another put in its place. Five pictures a minute may be taken with ease. If it is de-

Fig. 1. Outfit for Photographing Bread, Using the Sept Camera

sirable to have certain pictures developed before the complete roll has been used, the magazine gates may be closed and the exposed film taken out. By opening the gates again the machine is ready to operate.

Consecutive numbers are placed on the loaves and numbers corresponding to the photographic numbers are placed on the laboratory report containing the analytical and baking data. The finished prints are about an inch square and may be fixed to the laboratory report for a permanent record. The small print makes filing easy and does away with separate filing.

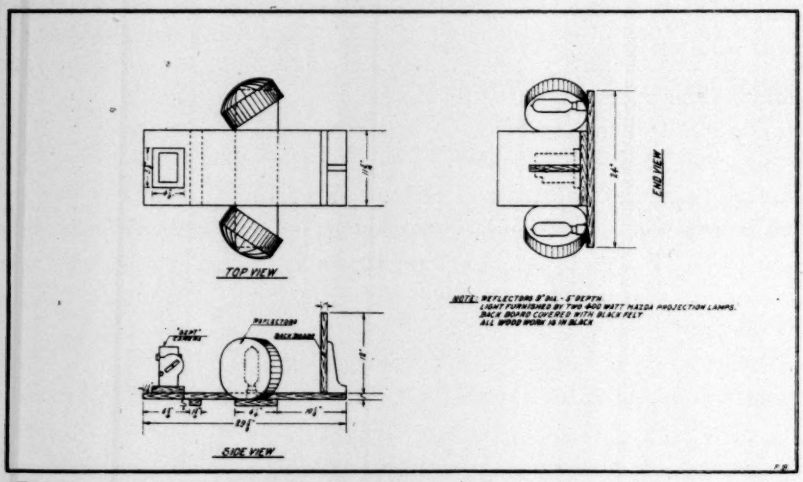

Fig. 2. Arrangement for Making Bread Record Photographs with the "Sept" Camera.

An enlarger has been used in connection with the camera, and exceptional results were obtained. The grain structure and general appearance are magnified to about the true size of the loaf. This brings out everything except the crumb color. The time required to make an enlargement is practically the same as is required to take the original picture, and the cost is very little, as the small print may be eliminated and the enlargement made from the negative.

The following are some of the advantages of this apparatus:

1. The cost for apparatus is nominal.
2. The developing and printing cost is small.
3. The time factor is almost eliminated in taking the pictures.
4. The illumination is uniform regardless of natural light.
5. Because of the size of the pictures they are easily filed.
6. Enlargements may be made from films at small additional cost.
7. The film can be developed positive for use in projection lantern.

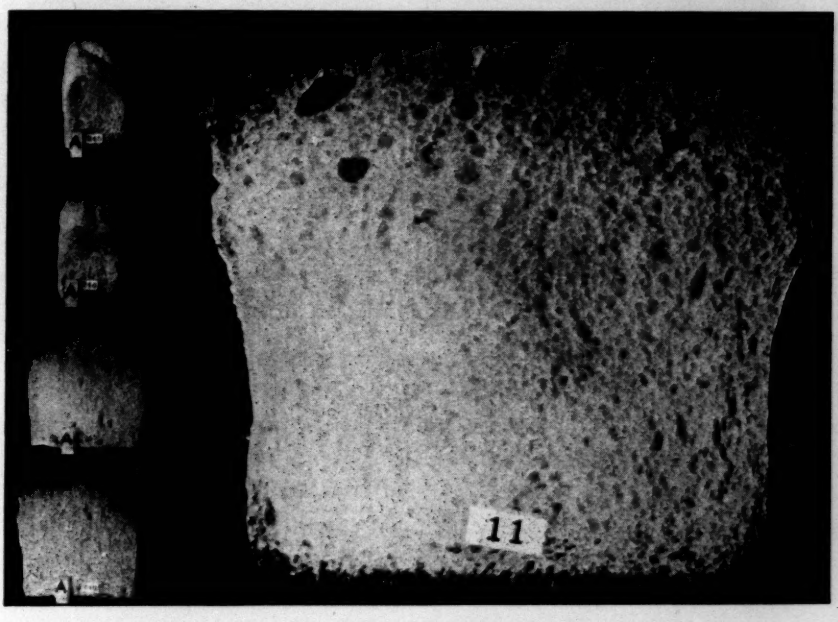

Fig. 3. Original Photograph and Enlargement

RELATION OF QUANTITY OF SODIUM SULPHATE TO TIME OF DIGESTION IN PROTEIN DETERMINATION

By C. G. HARREL AND J. H. LANNING
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(Received for publication October 20, 1928)

Coleman, Fellows, and Dixon[1] conclude that heat is the most important factor in protein digestion. They state that test results will vary with time of digestion and that this period of digestion depends upon the intensity of the heat. Their determinations embrace comprehensive data upon the Kjeldahl, Kansas City Protein Referee Board, and Gunning methods.

The following discussion relates only to the use of the Gunning method and its ability to check the Kjeldahl method. The burners employed are of a high heat classification, according to the methods of Coleman, Fellows, and Dixon, namely, 180 cc.

In all determinations, 20 cc. of 1.84 sulphuric acid, 0.15 gram of copper wire, and one gram of flour are employed. Table I gives the results when 7.5 grams of sodium sulphate is used with the time of digestion as the variable.

TABLE I

RESULTS WITH VARYING TIME INTERVALS

Time of digestion	Protein corrected
min.	%
15	10.11
20	10.13
24	10.15
28	10.17
32	10.11
36	10.17
40	10.17
45	10.19
50	10.29
60	10.32
70	10.35
80	10.39
90	10.39
110	10.41
120	10.43
130	10.37

*All results calculated to 13.5% moisture.

This table is in general agreement with the findings by the investigators mentioned.

[1]Cereal Chem. 2: 132-164.

The maximum percentage is obtained at the longest periods of digestion. This maximum, 10.43, is lower than the percentage obtained by the official Kjeldahl method, which was 10.54.

If heat is the most important factor in the protein determination, it is logical to assume that if the initial boiling point of the protein digestion mixture is raised, a shortening of the digestion period will follow, if burners of the same heat capacity are used.

Table II gives the data on the same flour, digesting for 35 minutes and varying the quantity of sodium sulphate. It is evident that as the sodium sulphate is increased the percentage protein increases.

TABLE II

RESULTS WITH 35-MINUTE DIGESTION AND A VARYING QUANTITY OF SODIUM SULPHATE

Sodium sulphate	Protein corrected
grams	%
6	10.15
7	10.17
8	10.35
9	10.37
10	10.47
11	10.49
12	10.51
13	10.51
14	10.53
15	10.49
16	10.53
17	10.55
18	10.54
19	10.53
20	10.53

In Figure 1 the results are represented graphically. When increments in sodium sulphate are made from 14 grams, the curve becomes parallel with the abcissa. The average value of the ordinate between 14 and 20 grams of sodium sulphate is 10.53, which agrees with 10.54 found by the official Kjeldahl method. This is the same flour from which the data in Table I were obtained. In Table II, when 14 grams of sodium sulphate was used with only 35 minutes time, digestion was more complete than in Table I, where 7.5 grams of sodium sulphate was employed with the time extended to 130 minutes.

This comparison reveals the importance of the initial sodium sulphate concentration, which is further emphasized in Table II when decrements in sodium sulphate are made, starting with 14 grams.

Tables III and IV contain the values of the individual determinations on a cake flour. The data compiled in the other tables are an average taken in a similar manner.

TABLE III

INDIVIDUAL RESULTS WITH 50-MINUTE DIGESTION PERIOD AND A VARYING QUANTITY OF SODIUM SULPHATE

Sodium sulphate	Determination number								Average protein
	1	2	3	4	5	6	7	8	
grams									%
6	7.94	7.96	8.06	8.06	8.06	7.98	7.98	7.98	8.002
7	8.02	7.98	8.02	8.02	8.06	7.98	8.06	8.02	8.020
8	8.02	8.02	8.18	8.06	8.07	8.06	8.09	8.02	8.065
9	8.02	8.14	8.14	8.06	8.06	8.02	8.14	8.06	8.080
10	8.14	8.06	8.09	8.09	8.14	8.06	8.09	8.09	8.095
11	8.18	8.09	8.09	8.08	8.08	8.09	8.09	8.08	8.097
12	8.14	8.22	8.14	8.09	8.22	8.14	8.22	8.18	8.168
13	8.22	8.18	8.09	8.22	8.22	8.18	8.09	8.22	8.177
14	8.14	8.22	8.14	8.22	8.18	8.22	8.14	8.18	8.180
15	8.22	8.14	8.18	8.14	8.22	8.18	8.22	8.22	8.190
16	8.22	8.20	8.18	8.18	8.14	8.22	8.18	8.19	8.189
17	8.26	8.18	8.18	8.26	8.22	8.26	8.18	8.22	8.220
18	8.26	8.22	8.26	8.18	8.18	8.18	8.22	8.18	8.210
19	8.22	8.22	8.18	8.24	8.22	8.18	8.18	8.26	8.212
20	8.24	8.22	8.18	8.18	8.22	8.22	8.24	8.18	8.210

Table III gives the data with a 50-minute digestion period. Figure 2, Curve A, gives the graphical presentation. Constancy in protein percentage is obtained with approximately 15 grams of sodium sulphate. It is interesting to note that altho this flour has a protein percentage lower than the flour in Table II, it required a greater quantity of sodium sulphate and a longer time for complete digestion.

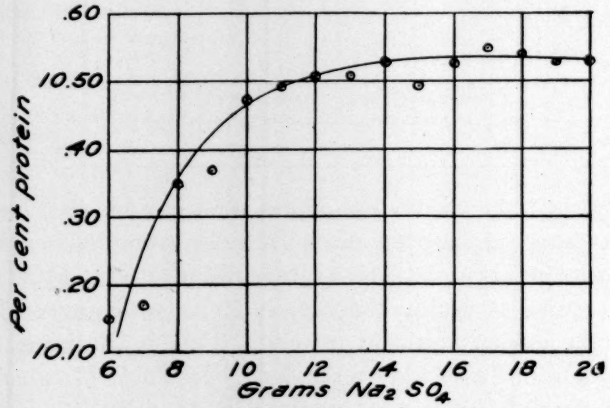

Fig. 1. A 50-Minute Kjeldahl Protein Digestion Curve with Dosage of Na_2SO_4 as the Variable

Table IV embodies data on the flour analyzed in Table III, but replacing the copper with 0.5 gram of red mercuric oxide.

TABLE IV

INDIVIDUAL RESULTS WITH 50-MINUTE DIGESTION PERIOD AND A VARYING QUANTITY OF SODIUM SULPHATE, MERCURIC OXIDE BEING THE CATALYST

Sodium sulphate	Determination number								Average protein
	1	2	3	4	5	6	7	8	
grams									%
0	7.78	7.94	7.86	7.94	7.86	7.80	7.86	7.94	7.872
1	8.10	8.06	8.02	8.05	8.09	8.03	8.06	8.06	8.058
2	8.10	8.10	8.18	8.10	8.06	8.10	8.10	8.10	8.105
3	8.14	8.14	8.14	8.09	8.16	8.14	8.12	8.14	8.133
4	8.16	8.17	8.16	8.16	8.14	8.14	8.15	8.19	8.159
5	8.16	8.17	8.17	8.16	8.17	8.18	8.19	8.16	8.170
6	8.18	8.17	8.15	8.16	8.20	8.15	8.19	8.17	8.171
7	8.16	8.20	8.18	8.15	8.18	8.20	8.23	8.15	8.181
8	8.18	8.17	8.18	8.20	8.16	8.17	8.19	8.18	8.179
9	8.14	8.20	8.13	8.22	8.18	8.19	8.18	8.17	8.176
10	8.20	8.18	8.22	8.17	8.23	8.20	8.20	8.19	8.198
11	8.22	8.18	8.21	8.17	8.22	8.22	8.20	8.21	8.203
12	8.20	8.21	8.17	8.21	8.17	8.21	8.20	8.20	8.196
13	8.21	8.20	8.22	8.16	8.20	8.24	8.20	8.20	8.203
14	8.24	8.20	8.21	8.20	8.21	8.16	8.20	8.19	8.201
15	8.20	8.21	8.20	8.18	8.11	8.22	8.20	8.21	8.198
16	8.20	8.19	8.20	8.19	8.20	8.18	8.20	8.23	8.198
17	8.22	8.20	8.21	8.18	8.20	8.20	8.19	8.22	8.202

After digestion, the mercury was precipitated with the necessary amount of sodium thiosulphate. In Figure 2, Curve B, it is observed that the curve becomes parallel with the abcissa at approximately 10 grams. Curve B intersects Curve A at a sodium sulphate concentration very close to 15 grams. With less than 15 grams of sodium sulphate, Curve A diverges from Curve B. From Curves A and B, the superiority of mercury as a catalyst and the importance of having a sufficiently high concentration of sodium sulphate when copper is the catalyst is verified.

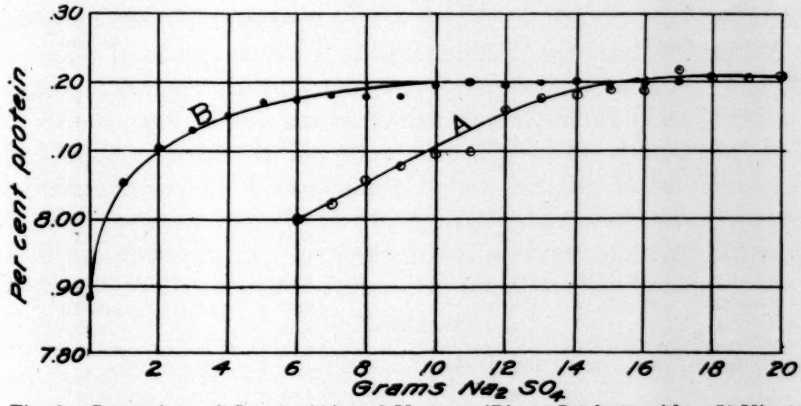

Fig. 2. Comparison of Copper (A) and Mercury (B) as Catalysts with a 50-Minute Digestion Period

In Table V are the data with a sodium sulphate concentration of from 6 to 20 grams, inclusive. In Figure 3, Curves A, B, and C represent the data obtained from 35, 50, and 80 minutes digestion periods, respectively.

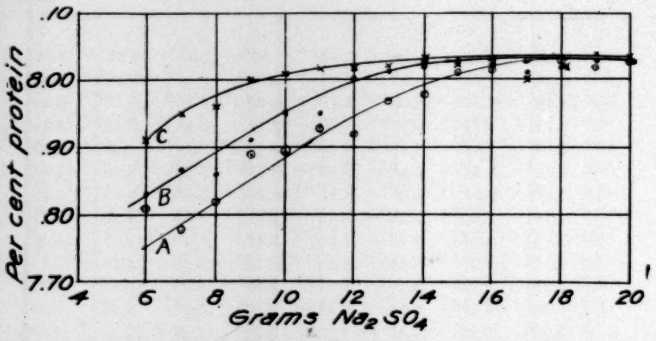

Fig. 3. Relation Between Na_2SO_4 Dosage, Time of Digestion, and Protein Percentage. Curve A, 35 Minutes; Curve B, 50 Minutes; and Curve C, 80 Minutes Digestion.

Curve A ceases to show successive increases in protein percentage with 17 grams of sodium sulphate, Curve B with 14 grams, and Curve C with 11 grams. As the digestion period is shortened, the quantity of sodium sulphate required to obtain a correct protein determination is increased. The official Kjeldahl method gave 8.02% protein on the flour reported in Table V.

Coleman, Fellows, and Dixon conclude that "Copper sulphate as a catalytic agent is of little value at low heat intensities," "heat intensities" referring to the capacity of the heating source.

TABLE V

RESULTS WITH A VARYING DIGESTION PERIOD AT DIFFERENT SODIUM SULPHATE CONCENTRATION

Sodium sulphate	35 min.	50 min.	80 min.
grams			
6	7.81	7.83	7.91
7	7.77	7.87	7.95
8	7.82	7.86	7.96
9	7.89	7.91	8.00
10	7.89	7.95	8.01
11	7.93	7.95	8.02
12	7.92	8.00	8.02
13	7.97	8.01	8.02
14	7.98	8.02	8.03
15	8.01	8.03	8.02
16	8.01	8.02	8.03
17	8.03	8.01	8.00
18	8.03	8.02	8.02
19	8.02	8.03	8.04
20	8.03	8.03	8.03

The data presented in this paper are in accord with the conception of the investigators mentioned. The data further point out that with the same heat source of high intensity, the success with which copper can be used as a catalyst with a given digestion period is dependent upon the initial ratio of sodium sulphate to acid. This ratio is continuously changing during digestion. The rate at which this occurs depends to some extent upon the characteristics of the individual apparatus, as refluxing and suction of the vapors, and nature and shape of flame.

Self[2] called attention to the point at which ammonia is lost from the digestion residue. The hazard of ammonia loss has since been stressed by several investigators. In our analysis, as high as 20 grams of sodium sulphate were used without loss. In hundreds of routine analyses, 15 grams of sodium sulphate and a 55-minute digestion period gave results that agreed with the official Kjeldahl method.

The error in the protein determination when insufficient sodium sulphate is used is difficult to detect because of its minuteness, and in some instances can be ascertained only by conducting several analyses. Referring to Table III, it is found that a number of determinations employing the same quantity of sodium sulphate, when such a quantity is insufficient, do not reveal this fallacy. This often leads the analyst to believe the result correct, because of the agreement of duplicate determinations.

This article involves no new findings in chemistry. It is a well recognized fact that the speed of most organic reactions is hastened by positive increments in the temperature of the reacting medium. This has partially been applied to protein determinations by specifying the heat capacity of the burner. The boiling point of the digestion mixture has been given little consideration, no doubt because of the difficulty of its determination.

The Gunning method, while unofficial, will continue to be used because it is more economical and involves fewer reagents. This paper has been prepared with the hope that it may induce chemists who use the Gunning method to pay more attention to the quantity of sodium sulphate employed. This quantity determines the initial boiling point of the digestion mixture. If the characteristics of the digestion apparatus are unknown, it is suggested that a series of determinations be conducted to find conditions that give complete digestion.

Summary

1. The weight of sodium sulphate used in the protein determination is a vital factor.

[2]Pharm. J. 188, pp. 384-385.

2. For a given heat source, the time required for complete digestion can be varied by changing the ratio of sodium sulphate to acid.
3. Low protein determinations can often be explained by failure to use sufficient sulphate when digesting with a given heat source and time interval.
4. Data from a large number of analyses prove the desirability of using a larger quantity of sodium sulphate if copper is the catalytic agent, than if mercury is used.

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REPORT ON CEREAL PRODUCTS TO THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

By J. A. LeClerc
General Referee, 1928
Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

(Received for Publication December 16, 1928)

A review of the work done by the A. O. A. C. on cereal products during the past 27 years was made, giving (1) the list of referees and their terms of service; (2) the various methods adopted by the association either as official or tentative, regarding flour, bread and alimentary pastes; and (3) a list of methods, much needed, but not yet fully considered by the A. O. A. C.

Suggestions were made to enlarge the scope of the A. O. A. C. work to include baked products other than bread. Reports from the associate referees were either presented in person or read by the general referee and include the following:

Sampling of flour, Associate Referee, H. Runkel.
Glutenin in flour, Associate Referee, M. J. Blish.
H-ion concentration, Associate Referee, C. H. Bailey.
Diastatic value of flour, Associate Referee, E. L. Tague.
Starch in flour, Associate Referee, L. H. Bailey.
Chlorine in bleached flour, Associate Referee, G. C. Spencer.
Sampling of bread, Associate Referee, L. H. Bailey.
Moisture in bread, Associate Referee, L. H. Bailey.
Lipoids and fat in bread, Associate Referee, Samuel Alfend.
Milk solids in bread, Associate Referee, L. H. Bailey.
Experimental baking tests, Associate Referee, M. J. Blish.

Unsaponifiable matter in fat of flour and alimentary paste, Associate Referee, Samuel Alfend.

Water soluble protein precipitated by 40 per cent alcohol, in alimentary paste, Associate Referee, Samuel Alfend.

Moisture in alimentary paste, Associate Referee, S. C. Rowe.

The general referee made numerous recommendations, most of which were approved by the committee. These appear below.

Besides all this, the general referee submitted a list of 59 references dealing with problems of interest to the cereal chemist and enumerated 20 patents relating to cereals and cereal products.

Recommendations of Referee

Flour

It is recommended—

(1) That the tentative method for sampling flour be adopted as official (first action).

Such action on this method was taken last year and the committee recommends that this method be kept in its present status until further work has been done.

Approved.

(2) That the associate referee continue studies on rapid methods of ashing flour, bread and alimentary pastes, giving special attention to the use of glycerol-alcohol mixture.

Approved.

(3) That the associate referee study the nature of the losses occurring when ash is fused.

Approved.

(4) That the F.A.C. method for the determination of unsaponifiable matter in fats and oils,[1] as modified and adopted as tentative for flour,[2] be retained as tentative.

The committee also recommends further study including collaborative work.

Approved.

(5) That the method for the determination of glutenin specified by the associate referee be adopted as tentative.

Approved.

(6) That study on the determination of hydrogen-ion concentration of flour be continued to include a comparison of the use of quinhydrone and antimony electrodes.

Approved.

[1] Assocn. Official Agr. Chem., 1926, 9:45: 1927, 10:35.
[2] Ibid., 1928, 11:37.

(7) That the study of methods for the determination and evaluation of the diastatic value of flour be continued.

Approved.

(8) That further study be conducted on the Seidenberg methods[3] and modifications thereof for the determination of chlorine in bleached flour.

Approved.

(9) That further study be made of the tentative method (Rask)[4] for the determination of starch in flour (bread and alimentary paste) and that the method be compared with the diastase method, as modified by Hartmann and Hillig.[5]

Approved.

(10) That before final action is taken on the recommendation considered last year to adopt the factor 5.83 for converting nitrogen into protein in wheat, consideration be given to the advisability of adopting this factor for scientific work only and retaining the old factor, 5.7, for all commercial transactions and regulatory work.

The committee does not favor the adoption of two conversion factors and would recommend further consideration of this matter before final action is taken upon the recommendation made last year for the adoption of the factor 5.83.

Approved.

(11) That the official method for the determination of water-soluble protein precipitable by 40 per cent alcohol[6] be dropped and the method as modified by the associate referee be made official (first action) and studied further.

Approved.

(12) That the Associate Referee on Bleaching Flour be directed to study methods to detect the use of benzoyl peroxide

Approved.

(13) That consideration be given by the appropriate associate referee to the study of certain foreign methods of analysis, especially those which are used by foreign governments in testing flour imported from this country.

Approved.

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[3] Ibid., 1925, 8:678.
[4] Assocn. Official Agr. Chem., 1928, 11:37.
Ibid., 1927, 10:108.
[5] Ibid., 1926, 9:482.
[6] Ibid., 1926, 9:40.

Baked Cereal Products

It is recommended—

(1) That the referee make further study of the tentative method for the sampling of bread[7] especial attention being paid to different types of bread.

Approved.

(2) That the tentative method for the determination of total solids in an entire loaf of bread[8] be made official (first action).

Approved.

(3) That the official method for the determination of fat (by acid hydrolysis) in flour[9] be adopted as tentative for baked products and that this method be compared with the present tentative method[10] for fat in baked cereal products.

Approved.

(4) That further study be made of the methods to determine lipoids in baked products.

Approved.

(5) That further work be done toward the development of methods for the determination of milk solids in bread.

Approved.

(6) That consideration be given to the development of methods for the estimation of rye flour in rye bread.

Approved.

(7) That the official method for the determination of chlorides in the ash of alimentary paste[11] be made official for baked cereal products (first action).

Approved.

(8) That the official methods for moisture determination in flour[12] be made official for air-dried baked cereal products (first action).

Approved.

(9) That the official method for the determination of crude fiber in flour[13] be made official for air-dried baked cereal products (first action).

Approved.

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[7] Ibid., 1926, 9:42.
[8] Assocn. Official Agr. Chem., 1926, 9:42.
[9] Ibid., 41.
[10] Methods of Analysis, A.O.A.C., 1925, 231.
[11] Methods of Analysis, A.O.A.C., 1925, 232.
Assocn. Official Agr. Chem., 1927, 10:34.
[12] Assocn. Official Agr. Chem., 1926, 9:39.
[13] Methods of Analysis, A.O.A.C., 1925, 225.

(10) That the official method for the determination of organic and ammoniacal nitrogen in alimentary paste[14] be made official for air-dried baked cereal products (first action).

Approved.

(11) That consideration be given to the study of baked products other than bread.

Approved.

(12) That the standard experimental baking test proposed by the associate referee[15] be adopted as tentative and that it be subjected to collaborative study.

Approved.

(13) That the associate referee make a recommendation next year on the subject of total solids in an entire loaf of bread by the 130° air oven and other rapid methods.

Approved.

Alimentary Paste

It is recommended—

(1) That the tentative method for collecting and preparing a sample of alimentary paste[16] for analysis be further studied with the view to making it official.

Approved.

(2) That the methods now official for the determination of moisture in flour[17] be adopted as official for alimentary paste (first action).

Approved.

(3) That further collaborative work be done with the tentative F.A.C. method for the determination of the unsaponifiable matter in the fats of alimentary paste.

Approved.

(4) That the tentative method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol[18] be dropped and that the modifications proposed by the associate referee be made official (first action).

"Place 20 grams of Sample B in an 8-ounce nursing bottle, add 100 cc. of water from a pipet, shake the bottle vigorously to prevent lumping of the sample, and add exactly 100 cc. more of water. Shake the stoppered bottle mechanically or by hand for 1

[14] Methods of Analysis, A.O.A.C., 1925, 232.
Assocn. Official Agr. Chem., 1926, 9: 39.
[15] Cereal Chem., 1928, 5:158
[16] Assocn. Official Agr. Chem., 1926, 9:43.
[17] Ibid., 39-40.
[18] Methods of Analysis, A.O.A.C., 1925. 225.

hour. The temperature of the water should not exceed 30°C.

"Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. The filtrate should be practically clear. Pipet 50 cc. of the filtrate into a 200 cc. nursing bottle. Add 0.6 gram of sodium chloride and dissolve. Add 0.2 gram of ignited asbestos, shake, and with constant agitation add 35 cc. of 95 per cent alcohol. Let stand overnight, then centrifugalize to pack the precipitate and asbestos. If the liquid is perfectly clear, pour it off and wash with two 20 cc. portions of 40 per cent alcohol, in each case shaking, centrifugalizing and decanting. If the liquid is not free of suspended matter, filter through a thin asbestos pad (0.1–0.15 gram) in a Gooch crucible, using light suction. Filter the subsequent washings also. Transfer the precipitate and asbestos from the nursing bottle to a Kjeldahl flask with the aid of a stream of water, add to it the mat in the Gooch crucible and determine the nitrogen by the Kjeldahl-Gunning-Arnold method, using about 40 cc. of 0.1 N acid to receive the distillate. Make a blank determination on the reagents and the asbestos."

Approved.

(5) That the official method for the determination of crude fiber in flour[19] be made official for alimentary paste (first action).

Approved.

(6) That the associate referee make a recommendation next year on the subject of air-oven methods for the determination of total solids.

Approved.

(7) That a report on the tentative acid-hydrolysis method for fat and the tentative method for lipoids and lipoid phosphoric acid (P_2O_5) in alimentary pastes, recommended as official (first action) last year, be made by the referee next year.

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19 Methods of Analysis, A.O.A.C., 1925. 225.